Renewal Assessment Report

beta-cyfluthrin

Bulldock EC 25

Volume 3 – B.9 Ecotoxicology data and assessment of risks for non-target species

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Co-Rapporteur Member State: Hungary

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When	What
31.03.2015	yellow draft

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B.9 Ecotoxicology data and assessment of risks for non-target species

Introduction

Ecotoxicological studies described in this document address data requirements specified in Commission Regulation No. 1107/2009 of 21 October 2009 and Commission Regulation No. 284/2013 of 1 March 2013. Experimental details of ecotoxicological studies done with the formulated product Bulldock 25 EC that also satisfy data requirements specified in Commission Regulation No. 283/2013 were included in Document M-CA; only the conclusions will be reported here in summary form.

Details of parameters, assumptions and calculations made for estimation of environmental concentrations used for calculations of Toxicity/Exposure Ratios (TER) are described in Vol_3CA8 and Vol_3CP_Bulldock_EC_25_B8.

The representative application pattern that drives the risk assessments in this re-submission is summarised in the following table. For information, these uses were assessed at Member State level after Annex I inclusion, and the risk to non-target species was concluded to be acceptable (including risk mitigation for the protection of aquatic organisms).

Table B.9.0-1 : Proposed use pattern of Bulldock 25 EC

Crop	Zone	Application method	Application timing e.g. BBCH	Number of applications	Minimum application interval (days)	Product application rate (L/ha)	Maximum individual application rate (g as/ha)
Winter wheat	N/C-EU	Foliar spray	49-75 (spring application 11-29 (autumn application)	2	14	0.3	7.5
Winter wheat	S-EU	Foliar spray	49-75 (spring application 11-29 (autumn application)	2	14	0.5	12.5
Spring wheat	N/C-EU	Foliar spray	10-75	2	14	0.3	7.5
Spring wheat	S-EU	Foliar spray	10-75	2	14	0.5	12.5
Potato	N/C-EU	Foliar spray	10-49	2	14	0.3	7.5
Potato	S-EU	Foliar spray	10-49	2	14	0.5	12.5
Tomato [1]	all zones	Foliar spray	9 up to PHI	2	14	0.7	17.5

[1] greenhouse use under protected growth

The potential risk from major metabolites has been considered during the first EU review (beta-cyfluthrin, see Monograph of 1996 and its Addenda of 2002). However, it has been concluded that there is no risk from the metabolites to non-target organisms.
To complete and underpin the earlier conclusion, new studies have been conducted with the major metabolites. The metabolites to which non-target organisms could be exposed are presented in the

table below.

Table B.9.0-2 Metabolites

Parent compound	Metabolite name	Compound found in	Maximal percentage of formation %
Beta-cyfluthrin	FPB-acid	Soil	12.7 / 63.9[1]
		Water	44.5 (total system)
		Sediment	24.3
	DCVA	Soil	40.5 / 75.7 1
		Water	47.6 (total system)
		Sediment	23.7
	FPB-aldehyde	Sediment	15.7

[1] maximum occurrences derived from laboratory aerobic / anaerobic study

B.9.1 Effects on birds and other terrestrial vertebrates

The risk assessment for birds was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)1, which provides a tiered approach to assess the effects of plant protection products on birds.

B.9.1.1.1 Effects on birds

B.9.1.1.2 Toxicity

Table B.9.1-1: Acute toxicity of beta-cyfluthrin, cyfluthrin, Bulldock EC 25 to birds

Species	LD_{50} (mg as/kg bw)	NOEL	(mg as/kg bw) Reference	Reliability
Beta-Cyfluthrin				
Bobwhite quail *Colinus virginianus*	> 2000, 3776 extrapolated with factor 1.888 according EFSA GD (2009)	2000	KIIA8.1.1/01 VB-027 [redacted], 1994 M-025760-01-1 R-19071	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin and in the corresponding list of endpoints valid
Cyfluthrin				
Bobwhite quail *Colinus virginianus*	> 2000, 3776 extrapolated with factor 1.888 according EFSA GD (2009)	2000	KIIA 8.1.1/03 426 [redacted] 1983 M-008638-01-1 R-19070	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin valid
Canary bird *Serinus canaria*	2000	< 125	KIIA8.1.1/10 EBBDL009 [redacted] 2012 M-442786-01-1 R-34708	New study, valid

Bulldock EC 25				
Bobwhite quail *Colinus virginianus*	> 2000 (formulation) > 58.6 (as,highest treatment group)	-	KIIIA 10.1.6/01 [redacted] 2010 M-367618-01-1 BAR/LD113	New study, valid
canary bird *Serinus canaria*	**170[1]**	**-**	**KIIA 8.1.1/11 Addy-Orduna, L 2011**	**open literature, reliable**
shiny cowbird *Molothrus bonariensis*	2234	--	KIIA 8.1.1/11 Addy-Orduna,L 2011	open literature, reliable
eared dove *Zenaida auriculata*	2271	-	KIIA 8.1.1/11 Addy-Orduna,L 2011	open literature, reliable
geometric mean LD_{50} = 1828 mg /kg bw				

[1]Relevant for risk assessment; value lower than geomean-LD_{50}/10 of 183 mg/kg bw for [4] tested species.

Table B.9.1-2: Long-term toxicity of cyfluthrin to birds

Species	Endpoint	NOEC/ NOAEC [mg as/kg feed]	NOEL/ NOAEL [mg as/kg bw/day]	Reference	Reliability
Beta-Cyfluthrin					
Mallard duck *Anas platyrhynchos*	Not available	269	Not available	[http://www.epa.gov/espp/litstatus/effects/redleg frog/2013/Cyfluthrin/assessment.pdf]	Not submitted, not validated
Cyfluthrin					
Mallard duck *Anas platyrhynchos*	Reproduction one generation, 24 weeks	250	23,8	KIIA8.1.4/03 508 [redacted] 1984 M-008671-01-1 R-19074	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin and in the corresponding list of endpoints supplemental
Mallard duck *Anas platyrhynchos*	**Reproduction one generation, 21 weeks**	**250**	**37.74**	**KIIA8.1.4/05 100359 [redacted] 1990 M-030237-01-1 R-19078**	**Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin** **valid**

Values in bold: Endpoints used for risk assessment

B.9.1.1.3 Acute oral toxicity to birds

KIIIA 10.1.6/01 (newly submitted with the dossier)

Author:	[redacted]
Title:	Acute Oral Toxicity to Northern Bobwhite Quail (*Colinus virginianus*) of beta-cyfluthrin EC 025G
Date:	22 April 2010
Doc ID:	M-367618-01-1
Report no.:	BAR/LD113

Guidelines:	U.S. EPA Pesticide Assessment Guideline § 71-1, Subdivision E, dated October 1982 with consideration of the recommendation of EPA Ecological Effects Guidelines OPPTS 850.2100 Avian Acute Oral toxicity Test, dated April 1996
GLP:	yes
Validity:	valid

Deviations: the study fulfils the requirements of the current OECD 223 guideline

Dates of experimental work: 2 February 2010 to 26 March 2010

Executive Summary
A laboratory study with the Bobwhite quail (*Colinus virginianus*) was conducted. After an acclimation period of 15 days, birds received a single dose of the test substance beta-cyfluthrin EC 025G in a gelatine capsule. The test consisted of two dosage groups and a control group. Nominal dosages used in the study were 1000 and 2000 mg formulation/kg bw. The control birds received a capsule only. Dosing was followed by a subsequent observation period of 14 days.

During the test mortality, behaviour changes, effects on food consumption and body weights were observed. Additionally, gross pathological changes were determined by necropsies. Body weights were measured individually 1 day prior to test start, on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group on days 3 to 7 of the test. *Post mortem* examination was carried out on all birds treated with the test item.

One female of the 2000 mg formulation/kg bw group died on day 6. In both test treatment groups effects such as soft excrement or diarrhoea were observed. With the exception of the control, females showed a slight decrease in body weight in the first week after application. All survivors recovered until test termination. At necropsy, the prematurely dead bird from the 2000 mg formulation/kg bw group showed strong signs of emaciation at liver, gallbladder, spleen, kidneys, pancreas and heart. All survivors showed no observable changes. Only one male at 1000 mg formulation/kg bw and 2000 mg formulation/kg bw showed an enlarged gallbladder. All validity criteria according to U.S. EPA Pesticide Assessment Guideline § 71-1, Subdivision E (1982) were fulfilled.

The acute oral LD_{50} for bobwhite quail exposed to beta-cyfluthrin EC 025G was determined to be > 2000 mg as /kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin EC 025G
Description:	Clear liquid
Lot/Batch #:	PF90225222
Purity:	2.93 % (26.11 g/L)

2. Vehicle and/or positive control: Gelatin capsula as vehicle and negative control

3. Test organisms:

Species:	Northern bobwhite quail (*Colinus virginianus*)
Age:	Adults, hatched on 18 September 2009 (4 ½ month old)
Weight:	170 - 211 g (1 day prior to test initiation)
Source:	[redacted]

Diet/Food: Standard rearing diet for quails (type. ssniff Wachtel-Zucht, Alleinfuttermittel für Wachteln V 6120-000 of the company ssniff Spezialdiäten GmbH, Soest, Germany) Food was offered *ad libitum* prior and throughout the study, except for a starvation phase 16 h prior to test start. Water was available at all times.

Acclimatisation: 15 days

4. Environmental conditions:

Temperature: 15.7 – 27.2 °C
Relative humidity: 13.2 – 46.2 %
Photoperiod: 11 hours light / 13 hours darkness

B. STUDY DESIGN AND METHODS

1. Experimental treatments:
Adult Bobwhite quails (5 males and 5 females per dosage) received a single oral administration with gelatine capsules. The test consisted of two dosage groups and a control group. Nominal dosages used in the study were 1000 and 2000 mg as/kg bw. The control birds received a gelatine capsule only.

2. Observations:
After administration birds were observed for 14 days. During the test, the following endpoints were observed: mortality, behaviour changes, effects on food consumption and body weights. Body weights were measured one day prior to test start, on days 7, and 14 of the test. Feed consumption was determined per cage of each dosage group and the control group on days 3 to 7 of the test. At the end of the test, gross pathological changes were determined by necropsies.
Post mortem examination was carried out on all birds treated with the test item.

3. Statistical calculations:
No statistical procedure for LD_{50} determination was possible since the LD_{50} was higher than the highest test dose (see next chapter).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.1-3: Effects of beta-cyfluthrin EC 025G on body weight and food consumption of Bobwhite quail

Beta-Cyfluthrin EC 025G [mg formulation/kg bw]			**Control**	**1000**	**2000**
Average body weight per animal [g] (± SD)					
Body weight	Day -1	Male	188 ± 8.1	190 ± 5.5	191 ± 6.1
		Female	186 ± 8.7	181 ± 17.1	188 ± 12.2
	Day 7	Male	187 ± 7.3	186 ± 6.5	184 ± 8.2
		Female	187 ± 12.3	180 ± 15.4	181 ± 12.1
	Day 14	Male	187 ± 6.7	189 ± 7.7	186 ± 9.5
		female	188 ± 15.1	183 ± 16.8	187 ± 7.0
Mean food consumption per animal [g/bird/day]					
Group Food consumption	Day 0 - 3		509	363	153
	Day 3 - 7		662	683	689
	Day 7 - 14		1126	1085	964
Mean food consumption per bird	Day 0 - 3		17 (n= 30)	12 (n = 30)	5 (n = 30)
	Day 3 - 7		17 (n = 40)	17 (n = 40)	18 (n = 38)
	Day 7 - 14		16 (n = 70)	17 (n = 70)	15 (n = 63)

n = number of bird-living days (e.g.10 birds × 3 days = n = 30

The LD_{50} value is given below based on nominal doses.

Endpoints	Beta-cyfluthrin EC 025G [mg formulation/kg bw]
LD_{50}	> 2000

B. OBSERVATIONS

One female of the 2000 mg formulation/kg bw group died on day 6. It stopped feeding after the application and did not excrete faeces, but only uric acid and was strongly emaciated at death. The excretion of uric acid, as an indicator of starving, was observed in all birds in the 1000 and 2000 mg formulation/kg bw groups on the day of dosing. One day later these symptoms were gone for the 1000 mg formulation/kg bw group. During the study single birds showed transiently impairment of the digestive tract (like soft excrement or diarrhoea). In the 2000 mg formulation/kg bw group the 9 surviving quails were free of any finding. Only on day 3 one of them showed soft excrement. This finding remained until the end of the test. Three further birds showed transiently soft excrement or diarrhoea in the further run of the study, but none of the survivors was affected in regards of behaviour or physical condition.
With the exception of the control, females showed a slight decrease in body weight in the first week after application. All survivors recovered until test termination. The food consumption was reduced between test start and day 3 compared to the control.

At necropsy, the prematurely dead bird from the 2000 mg formulation/kg bw group showed strong signs of emaciation at liver, gallbladder, spleen, kidneys, pancreas and heart. All survivors showed no observable changes. Only one male at 1000 mg formulation/kg bw and 2000 mg formulation/kg bw showed an enlarged gallbladder.

All validity criteria according to U.S. EPA Pesticide Assessment Guideline § 71-1, Subdivision E, dated October 1982 and OPPTS 850.2100 were fulfilled.

III. CONCLUSION

The acute oral LD_{50} for bobwhite quail exposed to beta-cyfluthrin EC 025G was determined to be > 2000 mg formulation/kg bw equivalent to > 58.6 mg as/kg bw. However, the maximum tested concentration in regard to the active substance is considerably lower than in the studies with the active substance. (please refer to Volume_3_CA B-9). Therefore, it is not possible to compare the toxicity of the active subance alone with the toxicity of the EC 25 formulation. However, an acute toxicity test on birds with the formulation is not a data requirement.

B.9.1.1.4 Higher tier data on birds

As all TER values are above the corresponding trigger values in the screening step assessment, no higher tier data on birds are necessary.

B.9.1.1.5 Effects on terrestrial vertebrates other than birds

Wild mammals will typically be exposed to dry residues of beta-cyfluthrin on their food items following the dilution and spraying of the formulated product. Since oral exposure is the main route of exposure, toxicity data for the active ingredients are used in preference to data from tests with the formulated substance. The toxicity of beta-cyfluthrin and the representative formulation to mammals is summarised below.

Mammalian acute oral and long-term reproduction studies have been carried out beta-cyfluthrin. A summary of these data and discussion on selected endpoints for risk assessment is given in Volume_3_CA_ B.9.2.2 for the active ingredient.

B.9.1.1.6 Toxicity

Acute oral LD_{50} value

Beta-Cyfluthrin
The derivation on the appropriate acute oral LD_{50} value is presented in Volume 3 CA B.9.2.2.1. The geomean **LD_{50} of 131.1 mg as/kg bw** is used for the acute risk assessment for mammals.
Bulldock EC 25
Please refer to Volume 3CP_Bulldock_EC_25_B.6.1.1..
The oral LD_{50} of Bulldock 25 EC (Beta-Cyfluthrin EC 25 g/L) is >300 mg/kg bw and <2000 mg/kg bw in rats. That corresponds to LD50 > 8.79 mg as/kg bw and < 58.6 mg as/kg bw. Therefore, the formulation Bulldock EC 25 mg/kg bw is more toxic to mammals than the technical material of beta-Cyfluthrin. This is considered in the risk assessment for Bulldock EC 25.

Reproductive NOAEL value

The EU agreed NOEC for the long-term and reproduction toxcitiy to mammals is 50 ppm. This corresponds to a **NOAEL** of 3.3 mg/kg bw/day. According the addendum on the monograph of beta-cyfluthrin (7 May 2002) this endpoint was based on two multi-generation studies with rats [[redacted] (1983), a 3- generation study and [redacted] 1996), a 2-generation study].
The re-evaluation of the studies reveal that the 3- generation study with rats [[redacted] (1983)] is not acceptable anymore. However, the 2-generation study with rats [[redacted] (1996)], is still assessed valid and applicable. The NOAEL of 3.3 mg/kg bw/day is confirmed. For details refer to Volume_3CA _B-6.6.1.

B.9.2 Risk assessment for birds and other terrestrial vertebrates

The worst case use pattern presented in Table B.9.0-1 for Bulldock EC 25 is derived from the GAP table.

The evaluation of the risk for birds was performed in accordance with the recommendations of the "Guidance Document on Risk Assessment for Birds and Mammals" (The EFSA Journal (2009) 7(12):1438).

B.9.2.1 Risk assessment for birds

The acute and reproductive risk assessment based on EFSA screening step for the critical use patterns relevant to the uses of beta-cyfluthrin is given in Table B.9.2-1 below.

Table B.9.2-1: Screening step TER calculations for birds using default worst case assumptions

Scenario	Appl. rate [kg as/ha]	Indicator species	Time scale	Shortcut value	MAF	f_{twa}	DDD	Endpoint [mg/kg bw/day]	TER
Wheat/potato	2 x 0.0075	Small omnivorous bird	Acute	158.8	1.2	1	1.43	92.2[1]	62.4
			Reproductive	64.8	1.4	0.53	0.36	37.74	105
Wheat/potato	2 x 0.0125	Small omnivorous bird	Acute	158.8	1.2	1	2.38	92.2[1]	38.5
			Reproductive	64.8	1.4	0.53	0.60	37.74	63

[1]Adjusted acceptability criterion = 1 please refer to Volume 1 section 2.9.1

The acute TER values as well as the reproductive TER values for the use of beta-cylfuthrin in wheat and potatoes were above 10 and 5, respectively. Therefore, tier 1 calculations are not needed.

Exposure via drinking water
There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.
The, 'Leaf scenario', is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain, or irrigation, water sufficiently to attract birds. Since none of the proposed crop uses fall into these categories, the leaf scenario does not apply to the use of Bulldock EC 25.

The puddle scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a heavy rainfall event follows the application of a pesticide to a crop, or to bare soil. This is therefore relevant for all uses of Bulldock EC 25and should therefore be assessed. According to the guidance, '*Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc > 500 L/kg)'*. The table below summarises the ratios for beta-cyfluthrin using both the acute and long-term endpoints.

Table B.9.2-2: Ratios of effective application rate to endpoints for beta-cyfluthrin following the use of Bulldock EC 25

K_{OC}	App. rate (g a.i/ha)	Acute endpoint (mg/kg bw)	Ratio of AR to acute endpoint	Long-term endpoint (mg/kg bw/day)	Ratio of AR to long-term endpoint	Ratio trigger
>>500	25	> 2000	0.012	37.74	0.66	3000

The resulting ratios fall below the corresponding trigger indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for beta -cyfluthrin and its metabolites.

B.9.2.1.1 Bioaccumulation and food chain behavior / secondary poisoning

According to the EC Guidance Document on Risk Assessment for Birds and Mammals, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

Beta-cyfluthrin has a log P_{OW} value of 5.9 and therefore the risk of biomagnifications in terrestrial food

chains has to be assessed.

Food chain from earthworm to earthworm-eating birds

The risk due to secondary poisoning via earthworms is based on a 100-g bird consuming 104.6 g worms per day. The concentration in earthworms is derived from the bioconcentration in earthworms, which is calculated from the K_{ow}, and the concentrations in soil.

The bioconcentration via the dry soil is calculated in accordance with the 'dry soil approach' in EFSA GD 2009. The calculations below are based on the worst case 21 day TWA PEC in soil from the representative use in potato.

Application rate (kg as/ha)	2 x 0.0125	
PECsoil 21 d TWA (mg as/kg)	0,023*	DT50 (soil) = 32,2 d
Kow	794,328	Log Pow = 5.9
Foc	0.02	default
Koc	112,004	Mean (n =)
BCF earthworm	4.26	BCF-worm/soil = (PEC-worm,ww / PEC-soil,dw)
PEC earthworm (mg as/kg)	0,099	PEC-worm = PEC-soil × BCF-worm
DDD birds (mg/kg bw/day)	0.104	DDD = PEC-worm × 1.05
NOEL (mg/kg bw/d)	37.74	Mallard duck
TER birds	**362.1**	**≥ 5, acceptable risk**

Application rate (kg as/ha)	2 x 0.0075	
PECsoil 21 d TWA (mg as/kg)	0,014*	DT50 (soil) = 32,2 d
Kow	794,328	Log Pow = 5.9
Foc	0.02	default
Koc	112,004	Mean (n =)
BCF earthworm	4.26	BCF-worm/soil = (PEC-worm,ww / PEC-soil,dw)
PEC earthworm (mg as/kg)	0,099	PEC-worm = PEC-soil × BCF-worm
DDD birds (mg/kg bw/day)	0.063	DDD = PEC-worm × 1.05
NOEL (mg/kg bw/d)	37.74	Mallard duck
TER birds	**603.4**	**≥ 5, acceptable risk**

In this worst case scenario the TER value is above the trigger of 5, indicating an acceptable risk to birds regarding secondary poisoning via earthworms. No further assessment is needed.

Food chain from fish to fish-eating birds

The risk due to secondary poisoning via fish to fish-eating vertebrates is based on a 1000-g bird consuming 159 g fish per day. The calculations risk from secondary poisoning to **fish-eating birds** was conducted in accordance with EFSA GD 2009.

Application rate (kg as/ha)	2 x 0.0125/2x0.0075	
RAC- aq (mg/L)	0.000000067	RAC tier 3 invertebrates
BCFfish	1822	Whole fish
BMF	2	Biomagnfication factor (relevant for BCF ≥ 2000)
PEC fish (mg as/kg)	0.000308	PECfish = PECsw*BCFfish*BMF
DDD birds (mg/kg bw/day)	0.000049	DDD = PECfish × 0.159
NOEL (mg/kg bw/d)	37.74	Mallard duck
TER birds	**1944382**	**≥ 5, acceptable risk**

In all scenarios the TER value is above the trigger of 5, indicating an acceptable risk to birds regarding secondary poisoning via fish. No further assessment is needed.

Biomagnification in terrestrial food chains

According the ADME studies (please refer to Volume_3CA _B-6.1) beta-cyfluthrin is not considered to be bioaccumulative, but is quickly excreted.
Therefore the risk of biomagnifications in terrestrial food chains is low.

B.9.2.2Risk assessment for mammals

The acute and reproductive risk assessment based on EFSA screening step for the critical use patterns relevant to the uses of beta-cyfluthrin is given in Table B.9.2-3 below.

Table B.9.2-3: Screening step TER calculations for mammals using default worst case assumptions

Scenario	Appl. rate [kg as/ha]	Indicator species	Time scale	Shortcut value	MAF	f_{twa}	DDD	Endpoint [mg/kg bw/day]	TER
Wheat/potato	2 x 0.0075	Small herbivorous mammal	Acute	118.4	1.2	1	1.07	> 8.79/ 131.1	>8.2/ 122.5
			Reproductive	48.3	1.4	0.53	0.27	3.3	12.2
Wheat/potato	2 x 0.0125	Small herbivorous mammal	Acute	118.4	1.2	1	1.78	> 8.79/ 131.1	>4.93/ 73.7
			Reproductive	64.8	1.4	0.53	0.45	3.3	7.3

With regard to the active substance only, the acute TER values as well as the reproductive TER values for the use of beta-cylfuthrin in wheat and potatoes were above 10 and 5, respectively. However, considering the higher toxicity of the product Bulldock EC 25 the acute TER values do not achieve the acceptability criterion of 10. Therefore, tier 1 calculations are represented in Table B.9.2-4 to Table B.9.2-7 below.

Table B.9.2-4: Tier I TER calculations for mammals in winter/spring wheat (2 x 7.5 g as/ha – 14d)

BBCH	generic focal species	shortcut value	MAF	DDD	Endpoint [mg/kg bw/day]	TER
BBCH 10-19	Small insectivorous mammal "shrew"	7.6	1.23	0.070	>8.79	**125.0**
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4		0.050		**175.9**
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9		0.378		**23.2**
BBCH 10-29	Small omnivorous mammal "mouse"	17.2		0.159		**33.1**
BBCH 30-39	Small omnivorous mammal "mouse"	8.6		0.034		**66.3**
BBCH ≥ 40	Small omnivorous mammal "mouse"	5.2		0.048		**109.6**

Table B.9.2-5: Tier I TER calculations for mammals in winter/spring wheat (2 x 12.5 g as/ha – 14d)

BBCH	generic focal species	shortcut value	MAF	DDD	Endpoint [mg/kg bw/day]	TER
BBCH 10-19	Small insectivorous mammal "shrew"	7.6	1.23	0.117	>8.79	**75.0**
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4		0.083		**105.5**
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9		0.631		**13.9**
BBCH 10-29	Small omnivorous mammal "mouse"	17.2		0.265		**33.1**
BBCH 30-39	Small omnivorous mammal "mouse"	8.6		0.133		**66.3**
BBCH ≥ 40	Small omnivorous mammal "mouse"	5.2		0.080		**109.6**

Table B.9.2-6: Tier I TER calculations for mammals in potatoes (2 x 7.5 g as/ha – 14d)

BBCH	generic focal species	shortcut value	MAF	DDD	Endpoint [mg/kg bw/day]	TER
BBCH 10-19	Small insectivorous mammal "shrew"	7.6	1.23	0.070	>8.79	**125.0**
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4		0.050		**175.9**
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9		0.378		**23.2**
BBCH 10-40	Large herbivorous mammal "lagomorph"	35.1		0.156		**56.3**
BBCH ≥ 40	Large herbivorous mammal "lagomorph"	10.5		0.097		**90.5**
BBCH 10-39	Small omnivorous mammal "mouse"	17.2		0.068		**129.4**
BBCH ≥ 40	Small omnivorous mammal "mouse"	5.2		0.048		**182.7**

Table B.9.2-7: Tier I TER calculations for mammals in potatoes (2 x 12.5 g as/ha – 14d)

BBCH	generic focal species	shortcut value	MAF	DDD	Endpoint [mg/kg bw/day]	TER
BBCH 10-19	Small insectivorous mammal "shrew"	7.6	1.23	0.117	>8.79	**75.0**
BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4		0.083		**105.5**
BBCH ≥ 40	Small herbivorous mammal "vole"	40.9		0.631		**13.9**
BBCH 10-40	Large herbivorous mammal "lagomorph"	35.1		0.541		**33.8**
BBCH ≥ 40	Large herbivorous mammal "lagomorph"	10.5		0.162		**54.3**
BBCH 10-39	Small omnivorous mammal "mouse"	17.2		0.113		**77.6**
BBCH ≥ 40	Small omnivorous mammal "mouse"	5.2		0.080		**109.6**

Applications in potatoes with 2 x 12.5 g as/ha (14d) cover application rates of 2 x 7.5 g as/ha (14 d).

The acute TER values for the use of beta-cylfuthrin in wheat and potatoes achieve the acceptability criterion of 10. Thus, the acute risk of Bulldock EC 25 to mammals is acceptable.

Exposure via drinking water
There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.
The, 'Leaf scenario', is relevant for mammals taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain, or irrigation, water sufficiently to attract birds. Since none of the proposed crop uses fall into these categories, the leaf scenario does not apply to the use of Bulldock EC 25.

The puddle scenario is relevant for mammals taking water from puddles formed on the soil surface of a field when a heavy rainfall event follows the application of a pesticide to a crop, or to bare soil. This is therefore relevant for all uses of Bulldock EC 25and should therefore be assessed. According to the guidance, '*Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc > 500 L/kg)'*. The table below summarises the ratios for beta-cyfluthrin using both the acute and long-term endpoints.

Table B.9.2-8: Ratios of effective application rate to endpoints for lambda-cyhalothrin following the use of Bulldock EC25

K_{OC}	App. rate (g a.i/ha)	Acute endpoint (mg/kg bw)	Ratio of AR to acute endpoint	Long-term endpoint (mg/kg bw/day)	Ratio of AR to long-term endpoint	Ratio trigger
>>500	25	131.1	0.19	3.3	7.6	3000

The resulting ratios fall below the corresponding trigger indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for beta -cyfluthrin and its metabolites.

B.9.2.2.1 Bioaccumulation and food chain behavior / secondary poisoning

According to the EC Guidance Document on Risk Assessment for Birds and Mammals, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

Beta-cyfluthrin has a log P_{OW} value of 5.9 and therefore the risk of biomagnifications in terrestrial food chains has to be assessed.

Food chain from earthworm to earthworm-eating mammals

The risk due to secondary poisoning via earthworms is based on a 10 g mammal consuming 12.8 g worms/day. The concentration in earthworms is derived from the bioconcentration in earthworms, which is calculated from the K_{ow}, and the concentrations in soil.

The bioconcentration via the dry soil is calculated in accordance with the 'dry soil approach' in EFSA GD 2009. The calculations below are based on the worst case 21 day TWA PEC in soil from the representative use in potato.

Application rate (kg as/ha)	2 x 0.0125	
PECsoil 21 d TWA (mg as/kg)	0,023*	DT_{50} (soil) = 32,2 d
Kow	794,328	Log Pow = 5.9
Foc	0.02	default
Koc	112,004	Mean (n =)
BCF earthworm	4.26	BCF-worm/soil = (PEC-worm,ww / PEC-soil,dw)
PEC earthworm (mg as/kg)	0,099	PEC-worm = PEC-soil × BCF-worm
DDD mammals (mg/kg bw/day)	0.127	DDD = PEC-worm × 1.05
NOEL (mg/kg bw/d)	3.3	rat
TER mammals	**26**	**≥ 5, acceptable risk**

Application rate (kg as/ha)	2 x 0.0075	
PECsoil 21 d TWA (mg as/kg)	0,014*	DT_{50} (soil) = 32,2 d
Kow	794,328	Log Pow = 5.9
Foc	0.02	default
Koc	112,004	Mean (n =)
BCF earthworm	4.26	BCF-worm/soil = (PEC-worm,ww / PEC-soil,dw)
PEC earthworm (mg as/kg)	0,099	PEC-worm = PEC-soil × BCF-worm
DDD mammals (mg/kg bw/day)	0.076	DDD = PEC-worm × 1.05
NOEL (mg/kg bw/d)	3.3	rat
TER mammals	**43.3**	**≥ 5, acceptable risk**

In this worst case scenario the TER value is above the trigger of 5, indicating an acceptable risk to mammals regarding secondary poisoning via earthworms. No further assessment is needed.

Food chain from fish to fish-eating mammals

The risk due to secondary poisoning via fish to fish-eating vertebrates is based on a 1000-g bird consuming 159 g fish per day. The calculations risk from secondary poisoning to **fish-eating birds** was conducted in accordance with EFSA GD 2009.

Application rate (kg as/ha)	2 x 0.0125	
RAC- aq (mg/L)	0.000000067	RAC tier 3 invertebrates
BCF_{fish}	1822	Whole fish
PEC fish (mg as/kg)	0.000308	$PEC_{fish} = PEC_{sw}*BCF_{fish}*BMF$
DDD mammals (mg/kg bw/day)	0.000044	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	3.3	rat
TER mammals	**190372**	≥ 5, acceptable risk

In all scenarios the TER value is above the trigger of 5, indicating an acceptable risk to birds regarding secondary poisoning via fish. No further assessment is needed.

Biomagnification in terrestrial food chains

According the ADME studies (please refer to Volume_3CA _B-6.1) beta-cyfluthrin is not considered to be bioaccumulative, but is quickly excreted.
Therefore the risk of biomagnifications in terrestrial food chains is low.

B.9.3 Effects on aquatic organisms

B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

KIIIA1 10.2.2.1/01

Author:	[redacted]
Title:	Acute toxicity of Bulldock to rainbow trout (*Salmo gairdneri*) in a flow-through test
Date:	5 September 1989
Doc ID:	M-055191-01-2
Report no.:	FF-272
Guidelines:	EEC 79/831 Method V C.I, OECD Guideline for Testing of Chemicals No. 203 (1984)
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD 203 guideline
Test material: Bulldock EC 25 (FCR 4545, beta-cyfluthrin), purity: 26.2 g/L, batch no. Fl. 103 according to 0004
Results: The LC_{50} (96h) of Bulldock EC 25 was 2.6 µg preparation/L with a 95 % confidence interval of 1.7 – 4.0 µg preparation/L. The lowest lethal concentration (LLC) was 4.0 µg preparation/L. The no observed effect concentration (NOEC) was 0.85 µg preparation/L.
Based on active ingredient this results in 96-h-LC_{50}: 0.08 µg as/L (95 % conf. lim.: 0.05 - 0.12 µg as/L), LLC: 0.12 µg as/L, NOEC: 0.03 µg as/L.
All calculations refer to nominal concentrations.

Conclusion: based on nominal values: LC_{50} (96 h) = 2.6 µg/L;
LC_{50} (96 h) = 0.08 µg as/L;

KIIIA1 10.2.2.1/02

Author:	[redacted]
Title:	Acute toxicity of Bulldock to golden orfe (Leuciscus idus melanotus) in a flow-through test
Date:	4.10. 1989
Doc ID:	M-055138-01-2
Report no.:	FO-1198
Guidelines:	OECD Guideline No. 203 of April 4, 1984
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD 203 guideline
Material/Study design
The acute toxicity of Bulldock (formulation: EC 025, active ingredient: Beta-cyfluthrin, 26.2 g/1) to Golden Orfe was determined in a 96-h-flow-through-test according to "OECD Guideline for Testing of Chemicals No. 203" dated 04.04..
The nominal concentrations tested were 5.0, 10.0, 20.0, 40.0 and 80.0 ng preparation/1 corresponding to 0.15, 0.29, 0.59, 1.18 and 2.36 ng asL/, a control without any additions and a solvent control group.

The active ingredient concentrations, measured in the watersamples, were between 17 - 390 % of the nominal values. But a precise analytical measurement of the active ingredient concentrations in the test media is extremely difficult at the very low concentrations of the test. Moreover the active ingredient is predominantly bound to suspended particles. Therefore a representative sampling is hardly possible. In those stock solutions, which are important for the results, the active ingredient concentrations were higher than 80 % of the nominal values during test duration, here the test substance was sufficiently stable.
Since the dosing system operated well, the results were related to the nominal concentrations of the test substance.

Results:
related to the preparation:
96-h-LC_{50}: 11.5 µg preparation/1 (95 % conf. lim.:9.3 - 14.2 µg preparation/L)
highest concentration without observable effect (NOEC): 5.0 µg preparation/L

related to active ingredient (beta-cyfluthrin, 26.2 g/1) in consideration of the density 0.89 g/ml of the preparation:
96-h-LC_{50}: 0.34 µg a.i.L/ (95 % conf. lim.: 0.27 - 0.42 µg a.i.L/),
NOEC: 0.15 µg a.i.L/.

Conclusion:
based on nominal values: LC_{50} (96 h) = 11.5 µg/L;
LC_{50} (96 h) = 0.34 µg as/L;

KIIIA 10.2.2.2/01 (newly submitted with the dossier)

Author:	Bruns, E.
Title:	Acute toxicity of beta-Cyfluthrin EC25A G to the waterflea Daphnia magna in a static renewal laboratory test system
Date:	14 June 2010
Doc ID:	
Report no.:	EBFRL008
Edition no.:	M-372834-01-1 (R-28699)
Guidelines:	OECD Guideline 202, (2004), U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982), EEC Directive 92/69/EEC, part C.2 (1992), OPPTS Guideline 850.1010 Draft (1996), modified, JMAFF 12 Nousan No. 8147 (2000)
GLP:	yes
Validity:	valid

Deviations: none

Dates of experimental work: 26 January 2010 to 21 March 2010

Executive Summary
The effects of beta-Cyfluthrin EC25A G on *Daphnia magna* were evaluated in a 2x24-hour static-renewal toxicity test. Thirty *Daphnia* (6 replicates of 5 animals per test beaker) per concentration were exposed to 4.27, 8.53, 17.1, 34.1, 68.3, 137 and 273 µg formulation/L nominal concentrations. In addition, 6 x 5 *Daphnia* were exposed to test water without test substance (blank control). Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test and 48 hours thereafter. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from the control and from all test concentrations at the beginning and at the end of each

renewal interval. The analysed test concentrations ranged between 87 % and 106 % (mean 94 %) of the nominal values. Due to the limited water solubility and stability of beta-cyfluthrin under test conditions, the corresponding concentrations of the aged test solutions at the end of each 24 hours exposure were distinctly reduced (mean: 52 %) of nominal. No contaminations of beta-cyfluthrin were detected in samples from untreated water control.
As the toxicity has to be attributed to the tested formulation as a whole, all results submitted by this report are related to nominal test concentrations of the formulated product.
However, endpoints based on the content of the active ingredient (nom) as well as on mean measured values were recalculated by the RMS.
All validity criteria according to the guideline OECD 202 were fulfilled.

The 48-h EC_{50} for *Daphnia magna* exposed to beta-Cyfluthrin EC 25 A G based on nominal concentration was 2.90 µg formulation/L with a 95 % confidence interval of 1.12 to 7.53 µg formulation/L (and 1.97 µg formulation/L mm).
This corresponds to EC_{50} = 0.0806 µg as/L (nominal) and to EC_{50} = 0.0547 µg as/L (mm).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin EC 25A G
Description:	Emulsifiable concentrate, clear colourless liquid
Lot/Batch #:	PF90225222
Purity:	Analysed content 2.93 % w/w (26.11 g/L)

2. Vehicle and/or positive control: fully defined, artificial water

3. Test organisms:

Species:	*Daphnia magna*
Age:	First instars (< 24 h old)
Source:	Laboratory bred
Loading:	5 organisms per vessel (100 mL glass beakers containing 50 mL test solution)

4. Environmental conditions:

Temperature:	20.3 to 20.6 °C
Photoperiod:	Light/dark 16/8 h
pH:	Start of the test: 7.9-8.1 End of the test: 7.9-8.1
Dissolved oxygen:	Start of the test: 8.2-8.5 mg O2/L End of the test: 8.2-8.3 mg O2/L
Conductivity:	581.0 / 583.0 µS/cm
Hardness:	231 mg/L CaCO3

B. STUDY DESIGN

1. Experimental treatments
The effects of beta-Cyfluthrin EC25A G on *Daphnia magna* were evaluated in a 48-hour static-renewal toxicity test. Thirty *Daphnia* (6 replicates of 5 animals per test beaker) per concentration were exposed to 4.27, 8.53, 17.1, 34.1, 68.3, 137 and 273 µg formulation/L nominal concentrations.
In addition, 6 x 5 *Daphnia* were exposed to test water without test substance (negative control). The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-

values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from the control and from all test concentrations at the beginning and at the end of the test.

3. Statistical calculations
For EC_{50} determination, a dose response relationship curve (displayed as sigmoid, shaped over the logarithm of the concentration) was modelled by Probit Analysis after Finney fitted by an iterative weighed linear regression according to the Maximum Likelihood principle which allows computation of EC_{50} and 95 % confidence limits for immobility rates if possible.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on nominal concentrations of beta-Cyfluthrin EC25A G, the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed.

probit analysis for data obtained after	EC_{50} µg formulation/L (nominally)	lower 95 % cl µg formulation/L (nominally)	upper 95 % cl µg formulation/L (nominally)
24 hours	14.7	11.4	18.9
48 hours	2.90	1.12	7.53

Analytical data: The accompanying chemical analysis of beta-cyfluthrin in the freshly prepared test solutions at start of each renewal interval revealed recoveries between 87 % and 106 % (mean: 94 %) of the corresponding nominal concentrations.
Due to the limited water solubility and stability of beta-cyfluthrin under test conditions, the corresponding concentrations of the aged test solutions at the end of each 24 hours exposure were distinctly reduced (overall final mean: 52 %) of nominal.
The mean recovery rate for the whole time of exposure calculated by the RMS was 67.91 %.
No contaminations of beta-cyfluthrin were detected in samples from untreated water control.
As the toxicity has to be attributed to the tested formulation as a whole, all results submitted by this report are related to nominal test concentrations of the formulated product.

Reference item: For quality control of the breeding stock, an acute non-GLP toxicity test was performed separately in February 2010 using the reference substance $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L).

The 24 hour EC_{50} of 0.73 mg/L, as determined in this test, meets the range defined by OECD 202 (0.6 mg/L - 2.1 mg/L).

B. OBSERVATIONS

No immobilities or other effects on behaviour occurred in untreated control. The immobilisation increases with increasing test concentration. At 34.1 µg formulation/L, all daphnids are immobilised after 48 h.
The measured values for the physical chemical parameters met the required range and yielded no deviation from guideline recommendations.

Table B.9.3-1: Toxicity to *Daphnia magna* (based on nominal concentrations)

Nominal test concentration [µg formulation/L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours		Number of immobile *Daphnia* after 48 hours	
		n	%	n	%
Control	30	0	0.0	0	0.0
4.27	30	0	0.0	18	60.0
8.53	30	6	20.0	22	73.3
17.1	30	19	63.3	26	86.7
34.1	30	26	86.7	30	100
68.3	30	29	96.7	30	100
137	30	29	96.7	30	100
273	30	30	100	30	100

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to beta-Cyfluthrin EC 25A G based on nominal concentration was 2.90 µg formulation/L with a 95 % confidence interval of 1.12 to 7.53 µg formulation/L. This corresponds to EC_{50} = 0.0806 µg as/L (nominal) and to EC_{50} = 0.0547 µg as/L (mm).

KIIIA1 10.2.2.3

Author:	Heimbach, F.
Title:	Growth Inhibition of Green Algae (*Scenedesmus subspicatus*) by FCR 4545 EC 025
Date:	20 July 1988
Doc ID:	M-055550-01-1
Report no.:	HBF/AL 44
Guidelines:	ISO-Guideline ISO/DIS 8692 (Algal Growth Inhibition Test) 1984 and OECD Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (1984).
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD 201 guideline
Test material: Bulldock EC 25 (FCR 4545, beta-cyfluthrin), as content: 3.23 %, batch no. Fl. 054 according to 0004
Results: Bulldock EC 25 was tested at seven concentration from 0.32 to 10 mg as/L. The EC_{50} determined for the growth of the biomass (E_bC_{50}) after 72 hours was 3.06 mg as/L and after 96 hours 2.86 mg as/L. The EC_{50} determined for the algal growth rate (E_rC_{50}) after 72 hours was 3.96 mg as/L and after 96 hours 3.68 mg as/L.
The 'no-observed-effect-concentration' (NOEC) was 1.0 mg as/L. The lowest concentration tested with signs of toxicity was 1.8 mg as/L.
All calculations refer to nominal concentrations.

Conclusions:
EbC50 (96 h) = 2.86 mg as/L; ErC50 (96 h) = 3.68 mg as/L, NOEC (96 h) = 1.0 mg/L

B.9.3.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

KIIIA1 10.2.6/01

Author:	Heimbach, F.
Title:	Extended laboratory study on effects and recovery of a *Daphnia magna* population in a water-sediment system after application of ^{14}C-Cyfluthrin EC 050 [redacted]
Date:	7 June 1999
Doc ID:	M-041214-01-1
Report no.:	HBF/EDM 04
Guidelines:	-
GLP:	yes
Validity:	Supplementary data

Guideline: No standard requirements

Test material: Cyfluthrin EC 050 [redacted], as content: 6.0 %, batch no. 233825429
Study Design: The effects of a 50 EC formulation were investigated with 14C-labelled cyfluthrin in an extended test (29 days) with sediment under static conditions.

Results: After 48 hours of exposure, Based on the nominal initial concentrations the following EC_X concentrations (48 hours) were found: an EC_{15}: of 0.13 µg as/L, and an EC_{50}: of 0.34 µg as/L were determined based on the nominal initial concentrations.
After 29 days, no effect on water flea populations had been observed at;
NOEC = 0.10 µg as/L (nominal),
a slight decrease of the population with recovery without insertion of juveniles at 0.32 µg as/L (nominal).

Conclusion: supplementary data

KIIIA1 10.2.6/02

Author:	Heimbach, F.
Title:	Comparative toxicity of ^{14}C-Cyfluthrin EC 050 to *Gammarus pulex* in water and in a water sediment system under static laboratory conditions
Date:	21 January 2000
Doc ID:	M-020399-01-1
Report no.:	HBF/SP 01-99
Edition no.:	R-19104
Guidelines:	-
GLP:	yes
Validity:	plausible (test system with water only used)

Test material: [cyclopropyl-^{14}C]Cyfluthrin (>98 % radiochemical purity) in EC 50 [redacted] formulation (54.2 g/L)

Results: *Gammarus pulex* was tested in two systems under static conditions. Young field collected animals were exposed either for 7 days (in a water/sediment system) or for 21 days (in water only). In the water plus sediment test system no mortality higher than 30 % was observed at days 2 to 7 at any concentration. Thus, an EC_{50} could not be calculated, but would be > 180 ng as/L (nominal initial concentration). Related to the analysed initial concentrations the EC_{50} was of > 118 ng as/L.

In the test system with water only, the calculated EC_{50}-values were 84 ng as/L (nominal initial concentration) on day 2, 61 ng as/L on day 4 ,43 ng as/L on day 7 and 21 ng as./L on day 21. Related to analysed initial concentrations the EC_{50}-values were 53 ng as/L on day 2, 35 ng as/L on day 4, 24 ng as/L on day 7 and 11 ng as/L on day 21. The NOEC (21d) based on adversely affected behaviour is 7.6 ng as/L based on analysed initial concentrations and 3.55 ng as/L based on analysed concentrations at day 4.

Due to the insufficient analytics and increase in measured concentrations in water between 4 and 7 days, it is only possible to calculate mean concentrations based on the extrapolation from 4 days to 21 days of the measured values. Therefore, information about the concentrations after 4 days was used to derive 21 days endpoints based on estimated geometric mean concentration.

$NOEC_{behavior}$(21 d) = 1.03 ng/L
$NOEC_{mortality}$(21 d) = 6.61 ng/L

Beta-cyfluthrin (adjusted):
$NOEC_{behavior}$(21 d) = 0.43 ng/L
$NOEC_{mortality}$(21 d) = 2.77 ng/L

Conclusion:
The study is considered plausible.
Results from the test system with water only were used to derive estimated mean measured $NOEC_{behavior}$ (21 d) = 0.43 ng/L.

B.9.3.3Further testing on aquatic organisms

KIIIA1 10.2.2.1/3 (newly submitted with the dossier)

Author:	[redacted]
Title:	Beta-cyfluthrin (Bulldock 025 EC). Toxicity to Rainbow trout *Oncorhynchus mykiss* in outdoor microcosms
Date:	14 December 2005
Doc ID:	
Report no.:	IRV 118/053116
Edition no.:	R-19589
Guidelines:	SETAC (1999). Guidance document on Higher Tier Aquatic Risk Assessment for Pesticides (HARAP)
GLP:	yes
Validity:	Supplementary data

Dates of experimental work: 19 April 2005 to 11 August 2005
Executive Summary
The purpose of the study was to determine the effects of two applications of beta-cyfluthrin on the

rainbow trout (*Oncorhynchus mykiss*) under realistic outdoor conditions. The study used shallow (water depth 30 cm) static outdoor microcosms that were intended to represent a static or slow flowing ditch. Beta-cyfluthrin (Bulldock 25 EC) was sprayed onto the water surface to mimic the spray drift route of entry. Nominal concentrations in the water column were 0.31, 0.63, 1.25, 2.5 and 5 µg beta-cyfluthrin/L. The application dates were 27 June and 11 July, 2005.

Acceptability:

The study is considered as not appropriate to address the acute or long-term risk on fish. Test organisms were too old to address adverse effects on earlier life stages of fish. They were also too big at the start of the test (average weight 10.3 g versus 0,96 g in acute laboratory test) to address appropriately the risk for fish, e.g. by testing effects on smaller fish as in acute laboratory tests. It is thus used as additional information to assess the acute toxicity to fish.

The No Observed Effect Concentration (NOEC) was 1.25 µg as/L (nom) corresponding with NOEC (4 d) = 0.169 µg/L. The LC_{50} was 5 µg as/L (nom.) corresponding with LC_{50} (4 d) = 0.366 µg/L based on mean measured values.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC (beta-cyfluthrin)
Lot/Batch #:	60111178
Purity:	25.2 g/L (measured)

2. Vehicle and/or positive control: Control: untreated

3. Test organisms:

Species:	Rainbow trout (Oncorhynchus mykiss)
Age:	When received (5 May 2005) the mean wet weight and fork length was 2.88 g and 6.3 cm respectively. 7 days (9 June) before application fish weight had been increased to average 10.3 g wet weight.
Source:	[redacted]
Diet/Food:	NutraTrout Fry AB02 fish food

4. Environmental conditions:

Temperature:	14.40 – 19.91 °C
Dissolved oxygen:	5.86 – 13.86 mgO_2/L
pH:	7.16 – 9.13

B: STUDY DESIGN AND METHODS

1. Test systems and design

The study was undertaken outdoors [redacted] during June-July 2005. The study was conducted using fibreglass microcosms each 1.8 m long x 0.9 m wide x 0.6 m deep. Microcosms contained sediment (upper 2.5 cm of natural lake sediment 4 % OC, on top of 10 cm of base clay sediment) and overlying water to a depth of 30 cm (water volume 486 litres). Fourteen microcosms were used (two for a preliminary analytical trial, and twelve for the main study). The microcosms for the main study were numbered 22, 23 (controls) and 26 to 35. Emergent macrophytes (including Burr-reed *Spaganium erectum*) were positioned on one side of each microcosm along the long axis. These

macrophytes were in an area of approximately one third of the width of each microcosm. Some submerged macrophytes were also present. Temperature, pH and dissolved oxygen were monitored during the study.
Protection of test systems and fish: Approximately seven days before the application of test material, batches of ten fish were added to the microcosms. Following the addition of fish, nylon nets were placed over the top of each microcosm to prevent loss of fish. A cover was placed over approximately 25 % of the area of the microcosm to provide shade in strong sunlight. In addition, each microcosm was protected by a sloping white corrugated perspex shelter to reduce heat transmission in strong summer sunlight and prevent entry of water during heavy rain. To prevent formation of anaerobic conditions, which could have compromised health of control fish, each microcosm was gently aerated. Fish were fed every day at 1 % of their wet-weight.

Application of test material, and water sampling:
The application dates were 27 June and 11 July, 2005
Before the application of the test material, the cover, shelter and netting were removed. Emergent macrophytes were cut at the water surface to minimise spray interception and cuttings were removed. Screens (1 m high) were erected around each microcosm to prevent loss of test substance during application. The solutions of the test material were applied using a spray lance fitted with a single nozzle. The test material was evenly applied to the entire water surface. Spray solutions were sampled for analysis (both before spraying, and from the nozzle). Depth integrated water samples (four, 500 ml each) were collected from each microcosm and pooled (total volume 2 litres). Aliquots (200 ml) were taken and added to dichloromethane (50 ml) and sodium chloride (20 g) in a polyethylene container. Samples were analysed by GC-MS. Water samples were taken at 1, 2, 4 hours and 1, 2 days after each application. Additional samples were taken from the 5 µg as/L treatment level (4, 8, and 14 days after each application). Sediment samples were taken for the 2.5 and 5 µg as/L test levels on day 14 and 28, and analysed.

2. Observations
Microcosms were inspected for mortalities or visible abnormalities in fish directly after application and at least at hourly intervals over the first five hours after each application. In addition, microcosms were inspected at least three times each day after application until the end of the study (28 days after the first application). Whenever severely affected fish were observed, they were placed in an open-top stainless steel mesh cage (30 cm high, 40 cm diameter, mesh size 5 mm) in the respective microcosm. This was to allow closer observation of the fish and to make sure dead fish could be retrieved for weighing. Fish that appeared to recover were released from the cage, and those that died were removed and their wet-weight determined. At the end of the test, the fish in each microcosm were removed, killed by immersion in an overdose of anaesthetic, and their individual fork lengths and wet-weights determined.

3. Statistical calculations
The Bartlett´s test was used to test for homogeneity of variance over treatment groups. The test was not significant at the 1 % level. A one-way analysis of covariance was therefore carried out on the mean weight at Day 28. The mean body weight at Day -7 was included as a covariate in the analysis. The covariate was not significant at the 10 % level so no adjustment was made to the group means. The treated groups were compared with the control group using the Williams´ test for a monotonic trend.

II. RESULTS AND DISCUSSION

Beta-cyfluthrin concentrations in spray solutions for the 1st application ranged from 90 % to 99 % of nominal and concentrations in spray-rates (solutions sampled from the spray nozzle) were 92 % to 107 % of nominal. Spray solutions for the 2nd application ranged from 74 % to 86 % of nominal, and spray-rates ranged from 83 % to 88 % of nominal.

Measured concentrations shortly after application
The following table gives the measured levels of beta-cyfluthrin in water column samples taken one

hour after each application.

Table B.9.3-2: Concentrations of beta-cyfluthrin in water column at start (estimated) and one hour after application (measured)

Nominal concentration (µg/L)	beta-Cyfluthrin [µg/L]	
	First application (% nominal)	***Second application- 14 days after the first application (% nominal)***
	measured at 1 hour after application	measured at 1 hour
0.31	0.310 (100)	0.211 (68)
0.31	0.297 (96)	0.297 (96)
0.63	0.623 (99)	0.493 (78)
0.63	0.584 (93)	0.383 (61)
1.25	1.199 (96)	0.851 (68)
1.25	1.043 (83)	0.678 (54)
2.5	2.393 (96)	1.628 (65)
2.5	2.049 (82)	1.235 (49)
5.0	4.477 (90)	2.956 (59)
5.0	4.762 (95)	2.699 (54)

Measured concentrations of beta-cyfluthrin one hour after the 1st application ranged from 82 % to 100 % of nominal. After the 2nd application, measured concentrations ranged from 49 % to 96 % of nominal one hour after application.

Measured concentrations following the 1st application are close to nominal.
For the 2nd application, most measurements were clearly below the nominal concentrations. In case of the three highest test concentrations, approx. the half of nominal concentrations was measured. Although microcosms represent a complex exposure matrix in which highly sorptive substances such as beta-cyfluthrin are likely to dissipate rapidly from the water column, the difference between the measured concentration after the first and after the second application isn't comprehensible. Mistakes during the second application process can't be excluded.

The geometric mean of measured concentrations during the first four days after the first application of 5 µg as/L was calculated below Table B.9.3-3 to compare the LC_{50} from this study with the LC_{50} determined in the laboratory study with rainbow trout ([redacted], 2006; please refer to Volume_3CA_B-9.3.1).

Table B.9.3-3: The calculation of the mean measured concentration (4 d) after the first application of the highest test concentration – 5 µg as/L (nom)

time	measured concentration (µg/L)
0d	4.6195
1d	0.3585
2d	0.1285
4d	0.0845
Geometric mean	**0.366**

Dissipation from the water column, after the first and second applications, was calculated using simple first-order kinetics and is given in Table B.9.3-4 below.

Table B.9.3-4: Concentrations of beta-cyfluthrin in water column at start (estimated) and one hour after application (measured)

Nominal concentration (µg/L)	$DT_{50\ diss}$ (hours)	
	First application	Second application (after 14 days)
0.31	9.3	2.2
0.31	3.1	1.7
0.63	6.5	1.6
0.63	7.1	2.1
1.25	2.9	2.0
1.25	3.9	3.1
2.5	3.7	2.4
2.5	5.5	5.1
5.0	4.7	2.9
5.0	5.7	3.3

Levels of beta-cyfluthrin in sediment
Beta-cyfluthrin was only found at low levels (<LOQ, 0.01 mg/kg) in one sample taken from Microcosm 32 treated at the nominal 2.5 µg/L on Day 28, and in three samples taken from microcosms treated at 5 µg/L (Microcosm 34 Days 14 and 28 and Microcosm 35, Day 14).

Effects on fish
At concentrations of 0.31, 0.63 and 1.25 µg as/L (nominal), no effects on fish were observed. Some sublethal effects were only observed at 2.5 µg as/L between 1.5 and 3 hours after the first application, but not thereafter and also not after the second application. At 5 µg as/L, the highest concentration tested, severe sublethal effects were seen starting one hour after the first application or 2.5 hours after the second application. Mortality (50 % after the first application) was seen in the highest concentration tested at 5 µg as/L, but in none of the other concentrations.

Table B.9.3-5: Mesocosm with fish - sublethal effects and mortality

Test concentration (µg as/L)	Observed effects
0.31, 0.63, 1.25	No effects
2.5	1st appln: Mild sub-lethal effects* in max 20 % of fish at any one time point during first 3 h. Followed by recovery. No effects from 3.5 h after appln onwards. 2nd appln: No effects.***
5.0	1st appln: Severe sub-lethal effects** in max 70 % of fish at any one time point. Some recovery. **50 % mortality.** 2nd appln: Severe sub-lethal** effects in 1 fish, with recovery***

* Mild symptoms were one or more of the following: Swimming close to surface, swimming in open water away from macrophytes for prolonged periods, loss of co-ordination being unable to maintain position in water for prolonged periods.
** Severe symptoms included one or more of the following: Loss or orientation (swimming upside down for short periods), hyperventilation, coughing, rolling over.
***Recovery of the active substance 1 hour after application deviated clearly from expected nominal values.

III. CONCLUSIONS

The No Observed Effect Concentration (NOEC) was 1.25 µg as/L (nom) corresponding with NOEC (4 d) = 0.169 µg/L (mean measured).

The LC_{50} was 5 µg as/L (nom.) corresponding with LC_{50} (4 d) = 0.366 µg/L based on mean measured values.

The study is neither appropriate to overwrite the acute toxicity endpoints derived from the laboratory

studies nor to address the long-term toxicity fish.
Reasoning:

1. Requirements for higher-tier studies

The fish-microcosm –study cannot be considered as tier III-tier study, since it neither considers inter-species interactions as only one fish species was tested, nor intra-species interactions as fish were fed according to their number and weight, excluding thus competition for food. Thus, this study is technically equivalent to a fish prolonged toxicity test OECD 204 but performed under more realistic conditions (sediment, litter, macrophytes etc), although excluding natural light since it was performed indoor with changed, more realistic surroundings.

2. Fish length and weight – strong deviations from the guideline OECD 204

For recommendations concerning test species, the guideline OECD 204 refers to the guideline for acute toxicity OECD 203. According to this, the recommended total fish length is 5.0 ± 1.0 cm.
In the available acute toxicity tests with *O. mykiss*, these recommendations are met:

Study	Endpoint	fish length	fish weight
[redacted] 1994; flow-through, KIIA 8.2.1/02	LC_{50} (96 h, flow-through) = 0.068 µg as/L NOEC (96 h, flow-through) < 0.039 µg as/L.	4.7 cm	0,96 g
[redacted] 1988;flow-through, KIIA 8.2.1/01	LC50 (96h, flow-through) = 0.089 µg as/L NOEC(96h, flow-through) = 0.053 µg as/L	5.94 ± 0.59 cm	2.26 ± 0.77 g
[redacted] 2006; static; KIIA 8.2.1/07	LC_{50} (96 h, static) = 0.359 µg as/L (mm) NOEC(96 h, static) = 0.068 µg as/L (mm)	5.7 cm	2.34 g

However, the weight of test organisms used in the "microcosm" fish study ([redacted] 2005; KIIIA1 10.2.2.1/3) was 10.3 g and was thus about 4 to 10 times higher than in the acute laboratory studies ([redacted] 1994; flow-through, KIIA 8.2.1/02, [redacted] 1988; flow-through, KIIA 8.2.1/01 [redacted] 2006; static; KIIA 8.2.1/07). In regard to the recommended length, the two latter studies are already in the upper range of the guideline's recommendation. Consequently, test fishes with a weight of 10.3 g clearly fail the recommendation concerning acute as well as prolonged toxicity testing.

3. Differences in the sensitivity of smaller fish and earlier life stages

3.1. A slightly higher sensitivity to smaller test organisms in terms of mortality is observed when comparing both acute toxicity flow-through tests ([redacted] 1994; flow-through, KIIA 8.2.1/02 and [redacted] 1988; flow-through, KIIA 8.2.1/01) (i.e. 96h LC 50 of 0.068 µg/L , mean length of 4,7 cm versus 96h LC 50 of 0.089 µg/L ,mean length of 5,9 cm).
3.2. The endpoint determining the $RAC_{chronic\ fish}$ is derived from the ELS -study with *O. mykiss* ([redacted] 1985; KIIA 8.2.4). In this study, strong effects on the number of early swim ups (reduction of 90 %) and on total survival of juvenile test organisms (100 % mortality) were observed at the highest test concentration (0.16 µg Cyfluthrin/L mm). Strong effects on the total survival of juvenile test organisms (48 % mortality at 0.0848 µg/L and 34 % at 0.0318 µg/L; fish weight at the test end in control group was 435 mg) were measured at concentrations as low as 0.0318 µg/L (mm). Pronounced effects (reduced by 40 %) on the mean body weight were observed at even lower concentration: at 0.0177 µg/L (mm). Thus, it cannot be excluded that toxic effects are mainly attributed to the exposure of fish embryos (fish eggs) within the hatching stage. The molecular weight of Cyfluthrin – Isomers is low enough (434.29) to penetrate the fish egg. Furthermore, the metabolism of detoxification is not developed yet or significantly slower than in more developed juvenile or adult fish. Considering the comparatively high potential of Cyfluthrin for bioaccumulation (especially absorption), it can

be assumed that the substances accumulates in the fish egg. Analytical measurements of Cyfluthrin concentrations in the tissues of the test organisms in the FLC study support this assumption (. 1990; KIIA 8.2.5**)**. Concentrations of 16 – 21 µg as Cyfluthrin /kg egg tissue were found. Consequently, hatched fish might be already affected by sublethal stress although they were not reduced in their number. Due to these disturbances, the total number of swim up larvae is strongly reduced at the highest test concentration of 0.16 µg Cyfluthrin/L. At lower concentrations, these effects manifest in terms of total survival and body weight. At the second lowest measured test concentration of 0.017 µg/L, total survival was not affected, but the mean final body weight of the fish remained significantly reduced compared to the controls (i.e. 262 mg versus 435 mg).
The final body weight in the control group of the ELS- study was 0.435 mg/L and thus, 23 times lower than in the submitted "microcosm" study (2005; KIIIA1 10.2.2.1/3)
3.3. Effects on early life stages of fish were also observed in the FLC study with *P.promelas* (1990; KIIA 8.2.5**)**. Significant effects on F1 –egg hatchability were observed at 0.29 µg/L. Furthermore, the F0 and F1 post hatch survival was significantly reduced at day 7 – day 60 post hatch (58 % survival) and day 60 – day 120d post hatch (80 % survival), whereas no adverse effects on survival were observed at later test periods (with fish at older life stages) when exposed to 0.29 µg/L. Thus, younger life stages are obviously more sensitive than older life stages.
This conclusion is also in accordance with results determined in a FLC study testing the toxicity of the pyrethroid lambda-Cyhalothrin to *P.promelas* ((1990); please refer to RAR December 2013; B.9.2.2.3). In this study effects on the F1 hatchability were found at the highest test concentration. Survival of 28 d and 56 d old F0 larvae was reduced at the highest test concentration, survival of 28 d and 56 d old F1 larvae was even reduced at the second highest test concentration. In contrast, no effects on survivability were found in adult fish (150 – 300d old organisms).
Endpoints determined from FLC study with Cyfluthrin (1990; KIIA 8.2.5**)** are higher than endpoints from the ELS study (1985; KIIA 8.2.4). This is attributed to a generally higher sensitivity of O.*mykiss compared to P. promelas* to beta-Cyfluthrin, i.e. 3.3 times more sensitive in terms of acute effects on mortality, as supported by the following data from acute laboratory tests (performed with a comparable test design):
P. promelas (LC_{50} =1.18 µg/L; mm, static laboratory test; KIIA 8.2.1/10)
O. mykiss (LC_{50} =0.359 µg/L; mm, static laboratory test; KIIA 8.2.1/07)

In terms of chronic effects, the lower sensitivity can be concluded when directly comparing effect thresholds in the FLC-test (with *P. promelas;* 1990; KIIA 8.2.5) and ELS-test (with *O. mykiss;* 1985; KIIA 8.2.4)):
The NOEC determined in the FLC study is 0.14 µg/L. Neither effects on mortality of adult fish, nor effects on hatchability, the survival of juvenile life stages and growth were observed at this concentration. As this no effect concentration is almost equivalent with the highest test concentration in the ELS tests with rainbow trout causing 90 % reduction of swim ups and 100 % total mortality, a higher sensitivity of *O.mykiss* compared to *P. promelas* can be concluded.
The information is thus used as additional information to assess the acute toxicity to fish.

Microcosm studies

KIIIA1 10.2.3/03

Author:	Heimbach, F.
Beta	Biological effects and fate of Cyfluthrin EC 050 in outdoor microcosm ponds
Date:	15 March 2000
Doc ID:	
Report no.:	HBF/BT 02
Edition no.:	M-029184-01-1 (R-19090a)

Guidelines:	OECD Guidance Document "Freshwater Lentic Field Tests", July 1996 (Draft) andGuidance Document on Testing Procedures for Pesticides in Freshwater Microcosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991)
GLP:	yes
Validity:	Plausible, used to derive the tier 3 RAC (invertebrates)

This study (Heimbach 2000) was evaluated and accepted in the previous EU review under Directive 91/414/EEC [for detailed documentation please refer to addendum 1 (07 May 2002) to the DAR (1996)]. The endpoint for risk assessment was a No Observed Ecologically Adverse Effect Concentration (NOEAEC) of 50 ng as/L justified by the fact that no ecological adverse effects occured at concentrations below 50 ng as/L. The effects observed at 50 ng as./L were restricted to three species only. Effects at 10 ng/L were a temporary reduction of emerged numbers of Chaoboridae, which recovered. This NOEAEC of 50 ng as/L was used in combination with an Uncertainty Factor (or Assessment Factor AF) of 2, to derive an Ecologically Acceptable Concentration (EAC) of 25 ng as/L.

However, this study was assessed by UBA (evaluation 24/01/06) and a NOEAEC of 10 ng/L based on the recovery in terms of occurrence of emergence of *Chaoboridae* which was defined as most relevant endpoint. An AF of 4 was associated to this endpoint to derive a RAC of 2.5 ng cyfluthrin/L.

When re-evaluated according to the recommendations of the aquatic GD (EFSA 2014), it is proposed to derive both an ERO-RAC considering the recovery of a population of a sensitive species as in the previous evaluation of UBA, and also an ETO-RAC considering only negligible effects (based on a NOEC) (and not the recovery) on the population of the most relevant species.
In this re-evaluation, the ERO Option is thus set on the basis of the NOEAEC of 10 ng cyfluthrin /L as previously proposed in the UBA evaluation, leading to an ERO-RAC of 2.5 ng cyfluthrin /L, equivalent to 1.05 ng beta-cyfluthrin/L. However it should be noted that at this concentration of 10 ng/L, the cumulated number of emerged individuals at the end of the experiment is significantly decreased compared to the control, highlighting the fact that applying the ERO-RAC may not ensure a sufficient protection in terms of the sustainability of the population at risk.
The ETO-Option is set on the basis of the NOEC of the most sensitive endpoint which is the emergence of *Chaoborus*, but for this endpoint a NOEC can not be directly derived since strong effects occur at the lowest concentration tested, i.e. at 10 ng/L which can thus also not be considered as a LOEC. Indeed, there is negligible or no emergence after treatments until day 49 in all concentrations. By contrast, in controls adult Chaoboridae emerged in significant numbers from day 14. Therefore a 5 weeks delay in emergence is considered as relevant for the population of adults and for subsequent generations and thus for the sustainability of the population. To establish the ETO-RAC, a NOEC value < 10 ng/L has to be extrapolated.
As the concentrations tested differ of a factor 5, it is unlikely that the NOEC would correspond to only one concentration below the lowest tested only (i.e. 2 ng/L), since information on emergence of Chaoborus from another outdoor study (Jenkins, 2014) show that there is a factor of 10 between the concentrations eliciting non detectable effects to substantial effects (i.e. ca 56 %) in terms of cumulated emergence, over 14 days after a single treatment treatment of a late instar larvae or pupae. As the individuals are exposed at an earlier stage in teh study of in Heimbach 2000 (mean time to emergence for control individuals is 47 days after exposure (day 0) versus 1.34 days in Jenkins, (2014), it is assumed that they are more sensitive. Therefore the NOEC was calculated on the basis of a factor of 20 below the lowest concentration tested, i.e. a factor 10 for extrapolating from substantial effects to negligible effects and an additional factor 2 to account for a higher sensitivity of an earlier life stage. This is equivalent to a NOEC of 0.54 ng Cyfluthrin/L. The NOEC is then associated to a standard AF of 2 (AF of 2 to 3 is recommended in the aquatic GD (EFSA 2014)) to derive an ETO-RAC. The ETO-RAC of 0.25 ng cyfluthrin/L is equivalent to an ETO-**RAC of 0.105 ng beta-cyfluthrin/L**).

ETO-RAC is 0.105 ng beta-cyfluthrin/L
ERO-RAC is 1.05 ng beta-cyfluthrin/L

Applying the ETO-RAC is recommended since the ERO-RAC may not provide a sufficient protection level in terms of the sustainability of the population at risk.

KIIIA1 10.2.3/04

Author:	Hommen, U.; Heimbach, F
Title	Evaluation of an outdoor microcosm study on Cyfluthrin (report no. HBF/Bt 02 of March 15, 2000) for an aquatic risk assessment
Date:	28 April 2000
Doc ID:	
Report no.:	HBF/BT 02a
Edition no.:	M-032767-01-1 (R-19090b)
Guidelines:	-
GLP:	-
Validity:	-

The Evaluation of the microcosm study (Heimbach 2000/ KIIIA 10.2.3/09) by Hommen & Heimbach (2000) is documented in detail in addendum 1 (07 May 2002) to the DAR (1996) of the initial inclusion of beta-cyfluthrin into Annex I.
As the microcosm study by Heimbach (2000/ KIIIA 10.2.3/09) has been re-evaluated by the RMS (please refer to KIIA 10.2.3/09), the evaluation by Hommen and Heimbach (2000) is not summarised here.

KIIIA1 10.2.3/05 (newly submitted with the dossier)

Author:	Jenkins, W.R.
Title	**Beta-cyfluthrin (Bulldock 25 EC): toxicity to *Asellus, Crangonyx, Chaoborus* and *Cloeon* in outdoor microcosms**
Date:	02 December 2014
Doc ID:	
Report no.:	JDV0118
Edition no.:	R-34676
Guidelines:	-
GLP:	yes
Validity:	Additional information deriving the RAC (aquatic invertebrates)

This study was performed to assess the potential biological effects of the insecticide beta-cyfluthrin (Bulldock 25EC) on the abundance of populations of *Asellus, Crangonyx, Cloeon* and *Chaoborus* in outdoor freshwater microcosms (30 cm deep) and the emergence of adult *Cloeon* and *Chaoborus.*
The aims were to derive a No Observed Effect Concentration (NOEC) and to provide information on the fate of beta-cyfluthrin in aquatic systems. The microcosms are intended to represent edge-of-field ditch systems and the study design included the complete coverage of each microcosm by a walk-in enclosure formed of insect- proof netting. Hence, all emerging *Cloeon* and *Chaoborus* adults have been caught and enumerated in this study.

Methodology
Microcosms were established in January 2013; each comprised a layer of clay base sediment and an overlying layer of lake sediment and water adjusted to a depth of approximately 30 cm (nominal volume 486 Litres).
In February 2014 emergent macrophyte growth in each microcosm was adjusted to give an area along one edge occupying approximately 30 % of the width of each microcosm and twenty four microcosms were stocked with populations of *Asellus, Crangonyx, Cloeon and Chaoborus* collected either from the untreated central reservoirs of microcosm facilities or from field sites, for a preliminary trial and the main study. Qualitative and quantitative assessments of the numbers of invertebrates in

microcosms selected for the main study were made and numbers adjusted, either by additional collections or by exchanging colonisation samplers, in order to improve the similarity between populations.

A **preliminary trial** was conducted to assess the application, water sampling and analytical methodology. Beta-cyfluthrin was applied as an aqueous dilution of the formulation Bulldock 25EC.

In the **main study**, the numbers of *Asellus* and *Crangonyx* and *Cloeon* nymphs (i.e. larvae) were assessed on two occasions before the application of beta-cyfluthrin.
Invertebrates in each microcosm were sampled using two colonisation samplers containing leaves (predominantly Alder, *Alnus glutinosa*) placed on the sediment, two floating samplers containing *Elodea* and two Hester-Dendy samplers which were attached to the walls in the area of macrophyte growth. Any invertebrates that were potential predators of the taxa of interest were removed and placed into a microcosm that was independent of the main study.
Chaoborus larvae typically float motionless in the water column awaiting their zooplankton prey. These larvae (combined count of larvae and pupae) were assessed *in-situ* by counting those seen above white discs (616 cm2) and a white tray (1584 cm2) placed on the sediment surface, at approximately weekly intervals.
Beta-cyfluthrin was sprayed onto the water-surface to give nominal concentrations of 0.5, 1.6, 5, 16 and 50 ng as/L on two occasions at 2 weeks interval (6 and 20 May 2014). Each treatment was applied to three microcosms except the highest, which was applied to two microcosms. The control group comprised four untreated microcosms. In addition, beta-cyfluthrin was also applied to single microcosms containing stocks of leaves and *Elodea* at each of the nominal concentrations employed in the main study. This provided a source of pre-treated material for replenishing the leaf or *Elodea* substrate in the colonisation samplers in the main study.
The numbers of *Cloeon* nymphs in floating *Elodea* colonisers were assessed in the week after the first application of beta-cyfluthrin and subsequently, the numbers of *Asellus* and *Crangonyx* and *Cloeon* nymphs were assessed at three-weekly intervals beginning in the week after the second application. Any invertebrates that were potential predators were removed and transferred to a microcosm that was independent of the main study. The numbers of *Chaoborus* larvae in the water column were assessed at approximately weekly or twice-weekly intervals.
Emerging insects (except caddis, damselflies and dragonflies which were not included in the analysis and were released from the enclosures) were collected each week 3 times per week using a vacuum sampler and were preserved in 70 % alcohol for subsequent identification and enumeration. Insects were also trapped on four sticky traps placed in each enclosure until their use was discontinued on 9 July 2014. Sticky traps were replaced each week and covered with plastic film for storage and subsequent identification and enumeration.

Results

Main Study
Measured levels of beta-cyfluthrin one hour after the first and second applications (2 weeks later) expressed as % of nominal were between 80 and 120 % and thus expressed in nominal concentrations. Beta-cyfluthrin dissipated from the water column. Mean estimates of DT50 and DT90 calculated from measured levels at 16 and 50 ng as/L were 3.8h and 23.5h respectively after the first application and 1.1h and 9.9h after the second.

Asellus
Minimum Detectable Differences for abundance (MDDabu) were <70 % of the backtransformed mean at each treatment level on each sampling occasion. At 50 ng as/L, the mean count in the 1st post-application sampling (Day 21 i.e., 21 days after 1st application, 7 days after 2nd application) was statistically significantly lower than the control. There were no statistically significant differences from the control for later time points or lower treatment levels.
The LOEC and NOEC for *Asellus aquaticus* were 50 and 16 ng as/L, respectively. The response at 50 ng as/L can be allocated to Effect Class 2 (De Jong et al, 2008).

Crangonyx
The numbers of *Crangonyx* at 50 ng as/L (where the MDDabus ranged from 61 to 71 %) were statistically significantly lower than the control ($p \leq 0.001$) on every sampling occasion after the applications. At 16 ng as/L, numbers were statistically significantly lower than controls ($p < 0.05$) on Days 21 and 63 and at this treatment level, the increase in numbers seen in the control on Day 21 was absent. There were no statistically significant differences from the control at lower concentrations.
The LOEC and NOEC for *Crangonyx pseudogracilis* was 16 and 5 ng as/L, respectively. The response at 16 and 50 ng as/L can be allocated to Effect Class 5B (De Jong et al, 2008).

Cloeon
Before the 1st application, numbers of sampled mayfly nymphs fell as adults emerged. Subsequently the colonisers seemed to underestimate the total number of larvae present, as many more adults emerged than were enumerated as larvae in recent previous counts.
Because of this, counts of nymphs after application are only regarded as indicative, although they do suggest a lack of effects at 50 ng as/L.
There was high degree of variability in the numbers of adult *Cloeon dipetrum* that emerged from control and treated microcosms. Calculated MDDabus were predominantly ≥ 100 % on the first two sampling occasions after 1st application and between 78 % and 100 % when emergence was at its peak. Nevertheless, there appeared to be no treatment-related adverse effects since the highest total counts of adults in treatment groups (323, 416, 131, 218 and 223 at 0.5, 1.6, 5, 16 and 50 ng as/L respectively) all exceed the highest count in the control group.
Based on adult emergence, the NOEC for *Cloeon* was 50 ng as/L. The result at 50 ng as/L can be allocated to Effect Class 1 (De Jong et al, 2008).

Chaoborus
Counts of *Chaoborus* larvae were substantially reduced after the 1st application at 16 and 50 ng as/L. In the controls, adult emergence started before the 1st application. Hence, by the time of this 1st application larval counts were reduced. Nevertheless, the actual numbers of larvae and pupae present were sufficient to give a mean adult emergence of 32.3 per microcosm in the control during the first week after the 1st application. The reduced larval counts mean that these data can only be regarded as indicative. The larval counts suggest a lack of effect at 0.5 and 1.6 ng as/L.
Three species of *Chaoborus* emerged as adults from microcosms, the most abundant of which was *Chaoborus obscuripes*. Values of the Minimum Detectable Difference on abundance (MDDabu) for the total number of *Chaoborus* that emerged ranged between 56 and 80 % on Days 8, 15 and 22 and between 85 and 90 % on Day 29 towards the end of the emergence period. MDDabu values for *Chaoborus obscuripes* were less variable and ranged between 64 % and 77 % on Days 8, 15 and 22 and between 88 % and 93 % on day 29.
The total numbers of emerged *Chaoborus* and *Chaoborus obscuripes* were statistically significantly lower than controls at 16 and 50 ng as/L on Days 8, 15, 22 and 29. At 5 ng as/L, the total number that emerged was statistically significantly lower on Days 8, 22 and 29 and the numbers of *Chaoborus obscuripes* was significantly lower on Day 8. At the nominal 1.6 ng as/L, the total numbers that emerged were statistically significantly lower on Days 22 and 29. Although Days 22 and 29 were near the end of the emergence period the preceding counts also suggested an effect.

The LOEC and NOEC for *Chaoborus* were 1.6 and 0.5 ng as/L, respectively. The responses at 1.6 ng as/L and above should be allocated to Effect Class 4 (De Jong et al, 2008) because the enclosures prevented the possibility of recovery by immigration.

Conclusions
The abundant populations established, and methodology employed (including total enumeration of emerged adult *Chaoborus* and *Cloeon*), enabled quantitative assessment of effects on the four sensitive taxa studied.
The NOEC's for beta-cyfluthrin (two applications at a 14 day interval) were concluded to be:
Asellus aquaticus – 16 ng as/L
Crangonyx pseudogracilis - 5 ng as/L

Cloeon dipterum – 50 ng as/L
Chaoborus spp. – 0.5 ng as/L

Evaluation by the RMS and Conclusion:

Ecotoxicological effects
The study shows that *Chaoborus spp* is the most sensitive of the few species tested and emergence is the most sensitive endpoint. Indeed this endpoint is affected at 1.6 ng beta-cyf./L which thus delivers a NOEC of 0.5 ng beta-cyf./L .
The authors of the study derive a safe concentration for *Chaoborus* of 50 ng/L (100 x above the NOEC), as *Chaoborus* can quickly recover by recolonisation through flying. Therefore, they conclude that it is better to take a NOEC on another species, i.e. on the amphipods (*Crangonyx),* a species with a NOEC of 5 ng/L, to derive an EAC (i.e. 2,5 ng/L).
The fact that the risk based on *Chaoborus* would cover for tested species that may have a similar sensitivity and a lower potential for recovery - i.e. the surrogate species concept - is not appropriately taken into account. However, it could be argued that no other species in the field do present such a high sensitivity. But another type of limitation is that considering external recovery as sufficient for ensuring recovery of the population largely depends on the agricultural landscape in particular of the spatial vicinity of other undisturbed populations. Furthermore, *Chaoborus sp* in Central Europe is an univoltine species and this may thus entail the fast recovery of such populations.

In the view of these results, the RMS considers that a NOEC should be based on the emergence of *Chaoborus* spp. However there are several concerns in this study. They are the followings:

Shortcoming 1: A large proportion of the *Chaoborus* individuals emerged before the 1st exposure (mean time to emergence for control individuals was 1.34 days). This means that at the time of exposure, the remaining individuals were either at a late instar stage or at the pupae stage. It is well known that the (i) earlier larval stages are the most sensitive and (ii) that pupae do not feed and have low metabolic activity. As a result, it can be stated that the contamination is occurring at a stage the *Chaoborus* is the least sensitive. Such aspects of various sensitivity are not included in the default AF of 2-3 to apply on the most relevant endpoint of a mesocosm, as recommended in the aquatic GD (EFSA 2013). Micro- and mesocosm studies should be typically performed with sensitive populations (i.e. preferably either with young individuals or whenever relevant with mixed populations (i.e. containing eggs, larvae of different stages, pupae)) to have a good evaluation of the risk for the population of concern. In the mesocosm study of Heimbach 2000 on cyfluthrin, the treatment was applied earlier in comparison with the timing of emergence in control ponds compared to present study, performed in limited size microcosm mean time to emergence for control individuals was 47 days versus 1.34 days). As a result of these observations, can be stated that the NOEC of younger larval stages of *Chaoborus* would certainly be lower than 0.5 ng/L, although the variation of sensitivity of different larval instars of *Chaoborus* towards beta-cyfluthrin is not known. This study enables to state that NOEC < 0.5 ng beta-Cyfluthrin/L.

Shortcoming 2: A large proportion of the *Chaoborus* individuals emerged before the 1st exposure (mean time to emergence for control individuals was 1.34 days). Thus the number of adults emerged is very low at time of the 2nd exposure (day 14). This is mentioned in the report "In the controls, adult emergence started before the 1st application. Hence, by the time of this application larval counts were reduced ". Therefore, it is difficult to demonstrate statistically significant effects at low concentrations under such conditions. Furthermore, there is possibly higher variability between replicates towards the end of this emergence process than at the beginning.

Uncertainties related to the exposure of *Chaoborus* in test system: The authors report on *page 17 :" Chaoborus* was the most sensitive of all the exposed organisms. This is probably due to a combination of extreme high sensitivity and the tendency of the larvae and pupae to float near the water surface. The latter means that high exposure to a spray application made onto the water surface is likely (in

both studies the test item was sprayed onto the water surface)". This is not necessarily true since Chaoborus - at least some species - are close to the bottom during the day and come at the surface at night (see Haney et al 1990 cited by Rahel and Nutzman, 1994: "*Chaoborus* larvae undergo extensive dial vertical migrations in lakes with fish spending daylight hours near tor within bottom sediments and migrating to surface waters to feed on small zoopK after sunset"). They can also occurr in deep anoxic waters to avoid predators. In the case of this microcosm study, the treatment takes place in the day (see pictures) and thus if a dial migration occurs, then the larvae would be less exposed than other species. However under the artificial conditions (i.e. ponds relatively shallow (depth of 30 cm), absence of predators), it remains questionable if *Chaoborus* is differently exposed than other species. Therefore it is concluded that the exposure of *Chaoborus* is representative of those from other species.

Conclusion
The study shows that among the different populations tested - *Asellus, Crangonyx, Cloeon* and *Chaoborus*- in outdoor freshwater microcosms treated with beta-cyfluthrin, *Chaoborus* is the most sensitive organism tested, especially when considering emergence. The UBA analysis of data on emergence of *Chaoborus* led to a NOEC below 0,5 ng beta-cyfluthrin/L. An accurate value can not be derived.

Additional pond studies reviewed for Annex I listing of beta-cyfluthrin, but not considered relevant for approval renewal.
An overview of the previous studies is given below.

KIIIA1 10.2.3/06

Author:	[redacted]
	Baythroid - Pond study
Date:	30 June 1986
Doc ID:	M-059891-01-1
Report no.:	F-867962
Guidelines:	-
GLP:	yes
Validity:	not applicable

Dates of experimental work: 16 August 1985 to 11 December 1985

This study was not considered acceptable for the derivation of the tier 3 RAC (invertebrates) as ponds included fish.

KIIIA1 10.2.3/01

Author:	Heimbach, F.
	Biological effects and fate of FCR 4545 EC 025 (Bulldock) in experimental ponds
Date:	21 December 1989
Doc ID:	M-059813-01-1
Report no.:	HBF/VT 01
Guidelines:	-
GLP:	yes
Validity:	not applicable

This documented in the DAR (1996) of the initial inclusion of beta-cyfluthrin into Annex I.

It is not considered acceptable for the derivation of the tier 3 RAC (invertebrates) as ponds included fish.
Furthermore the study has several shortcomings:

- predominantly low population densities at the time of application
- insufficient analytics
- no NOEC derivable due to strong, long-lasting effects on numerous species

Guideline: Not available
Deviations: Not applicable
Dates of experimental work: 16 May 1988 to 06 September 1988

KIIIA1 10.2.3/02

Author:	Heimbach, F.
Beta	Biological effects and fate of FCR 4545 EC 025 (Bulldock) in artificial ponds
Date:	12 December 1990
Doc ID:	M-059808-01-1
Report no.:	HBF/MT 01,
Guidelines:	-
GLP:	yes
Validity:	not applicable

This documented in the DAR (1996) of the initial inclusion of beta-cyfluthrin into Annex I.
This study was not considered acceptable for the derivation of the tier 3 RAC (invertebrates) as it included fish in the ponds.

B.9.4 Risk assessment for aquatic organisms

B.9.4.1 Approaches and endpoints used for risk assessment for aquatic organisms

The approaches used to examine the potential risk of beta-cyfluthrin formulation Bulldock EC 25 to aquatic organisms followed recommendations given Aquatic Guidance Document (EFSA , 2013). The risk assessment followed a tiered approach. In the first tier, estimations of exposure are refined in a step-wise approach

In the higher tier (tier 2), the RAC for fish acute is derived by using the SSD approach (please refer to Volume 1,Section 2.9.3.1). The higher tier RAC (tier 3) for aquatic invertebrates is based on an overall assessment taking all valid laboratory studies as well as the microcosm studies Heimbach (2000) and Jenkins (2014) into account (please refer to Volume,1 section 2.9.3.2).

The ecotoxicological endpoints used by the RMS for lower tier risk assessment are shown in the table below. The rationale for selection of values is presented in in Volume 1 section 2..9.3 and the evaluation of individual studies in volume_3CA_B-9.3.

Table B.9.4-1: Endpoints used by the RMS for the lower tier acute and long term aquatic risk assessment of Bulldock EC 25

Group/Species	Test substance	Time-scale/test design	End point	Toxicity (µg/L)	Reference
Laboratory tests ‡					
Fish					
Oncorynchus mykiss	Beta-cyfluthrin	96 h/ flow-trough	LC_{50}	0.068 (mm)	KIIA8.2.1/02 103231 [redacted] 1994 M-056053-01-1 R-19086
Oncorynchus mykiss	FPB – acid (metabolite)	96 h/static	LC_{50}	4060 (nom)	KIIA 8.2.1/13 EBFRL003 [redacted] 2010 M-364414-01-1 R-27962
Oncorynchus mykiss	DCVA (metabolite)	96 h/static	LC_{50}	>14700 (nom)	KIIA8.2.1/05 515 [redacted] 1984 M-034724-01-1 R-19097
Oncorynchus mykiss	FPB – aldehyd (metabolite)	96 h/static	LC_{50}	792	KIIA8.2.1/06 502 [redacted] 1984 M-034806-01-1 R-19096
Oncorynchus mykiss	Bulldock 25 EC	96 h/ flow-through	LC_{50}	0.08 (as)	KIIIA1 10.2.2.1 [redacted] 1989
Oncorynchus mykiss	Cyfluthrin Beta-cyfluthrin adjustment	58 d ELS / flow-through	NOEC NOEC	0.01 (mm) 0.0042 (mm)*	KIIA 8.2.4 683 [redacted] 1985 M-008695-01-1 R-19088
Aquatic invertebrates					
Hyalella azteca [a)]	Cyfluthrin Beta-cyfluthrin adjustment	96 h / flow-through	EC_{50}	0.00055 0.000231*	KIIA 8.3.1.3/04 M-458228-01-1 Bradley, 2013
Daphnia magna	Beta-cyfluthrin	48 h/static-renewal	EC_{50}	0.105 (mm)	KIIA 8.3.1.1/03 D58707 Kimmel, 2014a M-481046
Gammarus pulex	Cyfluthrin formulation	21 d/static	$NOEC_{behaviour}$ (21d) Adjusted values	0.00043 (mm)	KIIIA110.2.6/01 (KIIA 8.3.1.3/05; KIIA 8.3.2.1/06) HBF/SP 01-99 Heimbach, 2000 M-020399-01-1 R-19104
Americamysis bahia	Cyfluthrin	96 h/flow-through	EC_{50} (4d) adjusted value	0.00082 (mm)	KIIA 8.3.1.3/03 808 Surprenant, 1987 M-027941-01-1

Americamysis bahia	Beta-cyfluthrin	96 h/flow-through	EC_{50} (4d)	0,0022	KIIA 8.3.1.3/01 106797 Machado, 1994a M-056044-01-1 R-34702
Americamysis bahia	Beta-cyfluthrin	96 h/flow-through	EC_{50} (4d)	0,0023	KIIA 8.3.1.3/02 106588 Machado, 1994b M-056064
Daphnia magna	FPB-acid (metabolite)	48 h/ static	EC_{50}	39300 (nom)	KIIA8.2.4.8/07 09 EBFRL002 Bruns, 2010 M-363182-01-1 R-27963
Daphnia magna	DCVA (metabolite)	48 h/static	EC_{50}	25000 (nom)	KIIA 8.3.1.1/04 505 Forbis and Burgess, 1984 M-034747-01-1 R-19099
Daphnia magna	FPB-aldehyde (metabolite)	48 h/static	EC_{50}	1300 (nom)	KIIA 8.3.1.1/05 504 Forbis and Burgess, 1984 M-034810-01-1 R-19098
Daphnia magna	Bulldock EC 25	48 h/static-renewal	EC_{50}	0.055 (mm)	KIIIA 10.2.1/04 Bruns, E., 2010
Americamysis bahia	Beta-cyfluthrin	28 d/ flow-through	NOEC	0.00041 (mm)	KIIA 8.3.2.1/04 EBFRL028 M-465880-01-1 Schwader, 2013
Daphnia magna	Beta-cyfluthrin	21 d/ static-renewal	EC10 NOEC	0,023 (mm) 0,025 (mm)	KIIA 8.3.2.1/03 D58718 Kimmel, 2014 M-480965-01-1 R-30152
Sediment dwelling organisms					
Chironomus riparius	Beta-cyfluthrin	28 d/ static/water – sediment system/spiked water	NOEC	0.4 (nom)	KIIA 8.5.2/02 D58720 Kimmel, 2014c M-481015-01-1 R-30154
Chironomus riparius	Beta-cyfluthrin	28 d/ static/water – sediment system/spiked sediment	EC10	170 µg/kg (nom)	KIIA 8.5.2/03 D58731 Kimmel, 2014d M-481037-01-1 R-30153
Chironomus riparius	FBC-acid	Toxicity is addressed by the study with the active substance.			

Chironomus riparius	FPB-aldehyde	Toxicity is addressed by the study with the active substance.			
Chironomus riparius	DCVA	Toxicity is adressed by alternative information replacing experimental studies according EFSA GD (2013). (KIIA 8.2.5.4/04).			
Algae					
Scenedesmus subspicatus	Beta-cyfluthrin	96 h static	E_rC_{50} E_bC_{50}	>2 >2	KIIA 8.4/01 HBF/AL 40 Heimbach, 1987 M-056512-01-1 R-19109
Higher plant [*Not required in compliance with Reg (EU) 544/2011 article 8(2:8)]*					
Lemna gibba	Cyfluthrin	7 d static-renewal	E_rC_{50}	> 0.84 (mean measured)	KIIA 8.6 M-437708-02-1 Banman et al. 2012

*regarding to the adjustment please refer to Vol. 3CA B.9.2.2 and B.9.2.4

[a)] The acute toxicity endpoint in aquatic invertebrates reported by the notifier was based on a study on *D. magna* (Kimmel 2014a). Since H. azteca has proven to be more sensitive the RMS considers this better suitable for risk assessment.

[b)] The chronic toxicity endpoint in aquatic invertebrates reported by the notifier was based on a study on *D. magna* (Kimmel, 2014b). Since *Americamysis bahia* has proven to be more sensitive the RMS considers this better suitable for risk assessment.

For further evaluation and justification of recommended endpoints see Vol.3 CA B-9.2 and Vol 1 Level 2.

Summary of Regulatory Acceptable Concentrations

Fish:

tier	acute	chronic
1	LC_{50} (4 d, *Oncorynchus mykiss*) = 0.068 µg/L AF (assessment factor) = 100 **RAC_{acute} = 0.68 ng/L**	NOEC (56 d ELS,*Oncorynchus mykiss*) = 0.0042 µg/L AF = 10 **$RAC_{chronic}$ = 0.42 ng/L**
2	SSD median HC_5 LC_{50} = 0.312 µg/L AF (assessment factor) = 9 **RAC_{acut} = 34.6 ng/L**	

Invertebrates:

tier	acute	chronic
1	EC_{50} (4 d, *Hyalella azteca, mm*) = 0.23 ng/L AF (assessment factor) = 100 **RAC_{acute} = 0.0023 ng/L**	NOEC (21 d, *Americamysis bahia*) = 0,172 ng /L AF = 10 **$RAC_{chronic}$ = 0,0172 ng/L**

2	Geometric mean calculated on the basis of 4 species of invertebrates effect values [based on mean measured concentrations (ng/L)] *Daphnia magna*: 2d LC_{50} = 75.9 ng/L (geomean of LC_{50} = 55 ng/L and 105 ng/L) *Americamysis bahia*: 4d LC_{50} = 2.25 ng/L (geomean of 2 values: 2,22 ng/L and 2,23 ng/L) *Gammarus pulex:* 4d LC 50 = 4,0 ng/L (mean of values for 2 and 7d) *Hyallela azteca*: 4d LC 50 = 0,23 ng/L **Geomean LC_{50}: 3.54 ng/L** AF = 100 **RAC_{acute} = 0.0354 ng/L**	In a weight of evidence approach the selection of the lowest endpoint from the three invertebrate species tested is possible. Accounting for the part of the species sensitivity an a reduced AF of 6 is applied: *Daphnia magna*: NOEC (21d) = 25 ng/L *A.bahia*: NOEC (21d) = 0,41 ng/L *Gammarus pulex*: NOEC (21d) = 0,43 ng/L Relevant endpoint is 0.41 ng/L; AF = 6 **$RAC_{chronic}$ = 0.068 ng/L**
3	based on an overall assessment taking into accout the outcome of microsom studies (Heimbach (2000; KIIIA1 10.2.3/03); Jenkins, W.R.(2014; KIIIA1 10.2.3/05) as well as results from laboratory studies **ETO-RAC is 0.105 ng/L**** **ERO-RAC is 1.05 ng/L**	

** recommended, see this volume section B.9.3.3

B.9.4.2Lower tier acute and chronic risk assessment

The acute and chronic TER values calculated by the RMS are presented in Table B.9.4-2 to Table B.9.4-9.
As beta-cyfluthrin is not volatile, exposure to surface water bodies by the representative use in tomatos in greenhouse is expected. Therefore, the risk to aquatic organisms is deemed to be acceptable.

Table B.9.4-2: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in spring application in winter cereals]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.03350	0.12700	**2.02985075**	**0.12537313**	**0.00689552**	**0.01223881**	11.9402985	1338.58268	59.7014925	25.0746269
D1/stream	0.04170	0.40000	**1.63069544**	**0.10071942**	**0.00553957**	**0.00983213**	9.59232614	425	47.9616307	20.1438849
D2/ditch	003880	0.25100	**1.75257732**	**0.10824742**	**0.00595361**	**0.01056701**	10.3092784	677.290837	51.5463918	21.6494845
D2/stream	0.03410	0.17500	**1.9941349**	**0.12316716**	**0.00677419**	**0.01202346**	11.7302053	971.428571	58.6510264	24.6334311
D3/ditch	0.03830	0.15700	**1.77545692**	**0.10966057**	**0.00603133**	**0.01070496**	10.4438642	1082.80255	52.2193211	21.9321149
D4/pond	0.00154	0.03450	**44.1558442**	**2.72727273**	**0.15**	**0.26623377**	259.74026	4927.53623	1298.7013	545.454545
D4/stream	0.03000	0.03260	**2.26666667**	**0.14**	**0.0077**	**0.01366667**	13.3333333	5214.72393	66.6666667	28
D5/pond	0.00168	0.03300	**40.4761905**	**2.5**	**0.1375**	**0.24404762**	238.095238	5151.51515	1190.47619	500
D5/stream	0.03320	0.04430	**2.04819277**	**0.12650602**	**0.00695783**	**0.0123494**	12.0481928	3837.47178	60.2409639	25.3012048
D6/ditch	0.03850	0.17900	**1.76623377**	**0.10909091**	**0.006**	**0.01064935**	10.3896104	949.72067	51.9480519	21.8181818
R1/pond	0.02500	0.20100	**2.72**	**0.168**	**0.00924**	**0.0164**	16	845.771144	80	33.6
R1/stream	0.00147	0.03550	**46.2585034**	**2.85714286**	**0.15714286**	**0.27891156**	272.108844	4788.73239	1360.54422	571.428571
R3/stream	0.03520	0.09630	**1.93181818**	**0.11931818**	**0.0065625**	**0.01164773**	11.3636364	1765.31672	56.8181818	23.8636364
R4/stream	0.02500	0.31100	**2.72**	**0.168**	**0.00924**	**0.0164**	16	546.623794	80	33.6
TER criterion			100	10	100	10	10	10	10	10

Table B.9.4-3: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) autumn application in winter cereals]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.03350	0.1450	**2.02985075**	**0.02896552**	**0.00689552**	**0.01223881**	11.9402985	1172.41379	59.7014925	25.0746269
D1/stream	0.04320	0.4520	**1.57407407**	**0.00929204**	**0.00534722**	**0.00949074**	9.25925926	376.106195	46.2962963	19.4444444
D2/ditch	0.03860	0.2210	**1.76165803**	**0.01900452**	**0.00598446**	**0.01062176**	10.3626943	769.230769	51.8134715	21.761658
D2/stream	0.03290	0.09070	**2.0668693**	**0.0463065**	**0.00702128**	**0.01246201**	12.1580547	1874.31092	60.7902736	25.5319149
D3/ditch	0.03810	0.1410	**1.7847769**	**0.02978723**	**0.00606299**	**0.01076115**	10.4986877	1205.67376	52.4934383	22.0472441
D4/pond	0.03270	0.0850	**2.0795107**	**0.04941176**	**0.00706422**	**0.01253823**	12.2324159	2000	61.1620795	25.6880734
D4/stream	0.00152	0.0340	**44.7368421**	**0.12352941**	**0.15197368**	**0.26973684**	263.157895	5000	1315.78947	552.631579
D5/pond	0.03530	0.1030	**1.92634561**	**0.0407767**	**0.00654391**	**0.01161473**	11.3314448	1650.48544	56.6572238	23.796034
D5/stream	0.001610	0.03530	**42.2360248**	**0.11898017**	**0.14347826**	**0.25465839**	248.447205	4815.86402	1242.23602	521.73913
D6/ditch	0.03850	0.1950	**1.76623377**	**0.02153846**	**0.006**	**0.01064935**	10.3896104	871.794872	51.9480519	21.8181818
R1/pond	0.02490	0.3480	**2.73092369**	**0.01206897**	**0.00927711**	**0.01646586**	16.064257	488.505747	80.3212851	33.7349398
R1/stream	0.00170	0.04780	**40**	**0.08786611**	**0.13588235**	**0.24117647**	235.294118	3556.48536	1176.47059	494.117647
R3/stream	0.0350	3.1180	**1.94285714**	**0.00134702**	**0.0066**	**0.01171429**	11.4285714	54.5221296	57.1428571	24
R4/stream	0.0250	0.3090	**2.72**	**0.01359223**	**0.00924**	**0.0164**	16	550.161812	80	33.6
TER criterion			**100**	**10**	**100**	**10**	10	10	10	10

Table B.9.4-4: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) spring application in spring cereals]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.03910	0.2450	**1.73913043**	**0.01714286**	**0.005907928**	**0.01048593**	10.230179	693.877551	51.1508951	21.483376
D1/stream	0.03350	0.1200	**2.02985075**	**0.035**	**0.006895522**	**0.01223881**	11.9402985	1416.66667	59.7014925	25.0746269
D3/ditch	0.03830	0.1600	**1.77545692**	**0.02625**	**0.006031332**	**0.01070496**	10.4438642	1062.5	52.2193211	21.9321149
D4/pond	0.00150	0.02770	**45.3333333**	**0.15162455**	**0.154**	**0.27333333**	266.666667	6137.18412	1333.33333	560
D4/stream	0.03130	0.0473	**2.17252396**	**0.08879493**	**0.007380192**	**0.01309904**	12.7795527	3594.08034	63.8977636	26.8370607
D5/pond	0.00170	0.0312	**40**	**0.13461538**	**0.135882353**	**0.24117647**	235.294118	5448.71795	1176.47059	494.117647
D5/stream	0.03240	0.0365	**2.09876543**	**0.11506849**	**0.00712963**	**0.01265432**	12.345679	4657.53425	61.7283951	25.9259259
R4/stream	0.02500	0.0316	**2.72**	**0.13291139**	**0.00924**	**0.0164**	16	5379.74684	80	33.6
TER criterion			**100**	**10**	**100**	**10**	10	10	10	10

Table B.9.4-5: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) application in spring cereals]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	**NOEC (µg/L)**	**EC_{50} (µg/L)**	**NOEC (µg/L)**	**NOEC (µg/L)**	**EC_{10} (µg/Kg)**	**E_bC_{50} (µg/L)***	**E_rC_{50} (µg/L)**
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.065100	0.408000	**1.04454685**	**0.01029412**	**0.003548387**	**0.006298**	**6.14439324**	416.666667	30.7219662	12.9032258
D1/stream	0.055800	0.200000	**1.21863799**	**0.021**	**0.004139785**	**0.00734767**	**7.16845878**	850	35.8422939	15.0537634
D3/ditch	0.063800	0.266000	**1.06583072**	**0.01578947**	**0.00362069**	**0.00642633**	**6.26959248**	639.097744	31.3479624	13.1661442
D4/pond	0.002490	0.046200	**27.3092369**	**0.09090909**	**0.092771084**	**0.16465863**	160.64257	3679.65368	803.212851	337.349398
D4/stream	0.052200	0.078800	**1.30268199**	**0.05329949**	**0.004425287**	**0.00785441**	**7.66283525**	2157.36041	38.3141762	16.091954
D5/pond	0.002830	0.051900	**24.0282686**	**0.08092486**	**0.081625442**	**0.14487633**	141.342756	3275.52987	706.713781	296.819788
D5/stream	0.054000	0.060800	**1.25925926**	**0.06907895**	**0.004277778**	**0.00759259**	**7.40740741**	2796.05263	37.037037	15.5555556
R4/stream	0.041600	0.526000	**1.63461538**	**0.00798479**	**0.005552885**	**0.00985577**	**9.61538462**	323.193916	48.0769231	20.1923077
TER criterion			**100**	**10**	**100**	**10**	10	10	10	10

Table B.9.4-6: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in spring application in winter wheat]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.05580	0.2110	**1.21863799**	**0.075268817**	**0.004139785**	**0.00734767**	**7.16845878**	805.687204	35.8422939	15.0537634
D1/stream	0.06950	0.6660	**0.97841727**	**0.060431655**	**0.003323741**	**0.00589928**	**5.75539568**	255.255255	28.7769784	12.0863309
D2/ditch	0.06460	0.4180	**1.05263158**	**0.06501548**	**0.003575851**	**0.00634675**	**6.19195046**	406.698565	30.9597523	13.003096
D2/stream	0.05680	0.2920	**1.1971831**	**0.073943662**	**0.004066901**	**0.00721831**	**7.04225352**	582.191781	35.2112676	14.7887324
D3/ditch	0.06380	0.2670	**1.06583072**	**0.065830721**	**0.00362069**	**0.00642633**	**6.26959248**	636.70412	31.3479624	13.1661442
D4/pond	0.002560	0.05750	**26.5625**	**1.640625**	**0.090234375**	**0.16015625**	156.25	2956.52174	781.25	328.125
D4/stream	0.050	0.05430	**1.36**	**0.084**	**0.00462**	**0.0082**	**8**	3130.75506	40	16.8
D5/pond	0.00280	0.0550	**24.2857143**	**1.5**	**0.0825**	**0.14642857**	142.857143	3090.90909	714.285714	300
D5/stream	0.05530	0.07380	**1.22965642**	**0.075949367**	**0.004177215**	**0.0074141**	**7.23327306**	2303.52304	36.1663653	15.1898734
D6/ditch	0.06410	0.2990	**1.06084243**	**0.065522621**	**0.003603744**	**0.00639626**	**6.24024961**	568.561873	31.201248	13.1045242
R1/pond	0.04160	0.3350	**1.63461538**	**0.100961538**	**0.005552885**	**0.00985577**	**9.61538462**	507.462687	48.0769231	20.1923077
R1/stream	0.002450	0.05920	**27.755102**	**1.714285714**	**0.094285714**	**0.16734694**	**163.265306**	2871.62162	816.326531	342.857143
R3/stream	0.05870	0.1610	**1.15843271**	**0.071550256**	**0.003935264**	**0.00698467**	**6.81431005**	1055.90062	34.0715503	14.3100511
R4/stream	0.04170	0.5190	**1.63069544**	**0.100719424**	**0.005539568**	**0.00983213**	**9.59232614**	327.552987	47.9616307	20.1438849
TER criterion			100	10	100	10	10	10	10	10

Table B.9.4-7: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in autumn application in winter wheat]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D1/ditch	0.055580	0.2420	**1.22346168**	**0.01735537**	**0.004156171**	**0.00737675**	**7.19683339**	702.479339	35.984167	15.1133501
D1/stream	0.07190	0.7530	**0.945758**	**0.00557769**	**0.003212796**	**0.00570236**	**5.56328234**	225.763612	27.8164117	11.6828929
D2/ditch	0.06430	0.3680	**1.05754277**	**0.01141304**	**0.003592535**	**0.00637636**	**6.22083981**	461.956522	31.1041991	13.0637636
D2/stream	0.05480	0.1510	**1.24087591**	**0.02781457**	**0.004215328**	**0.00748175**	**7.29927007**	1125.82781	36.4963504	15.3284672
D3/ditch	0.06350	0.2350	**1.07086614**	**0.01787234**	**0.003637795**	**0.00645669**	**6.2992126**	723.404255	31.496063	13.2283465
D4/pond	0.05450	0.1420	**1.24770642**	**0.02957746**	**0.004238532**	**0.00752294**	**7.33944954**	1197.1831	36.6972477	15.412844
D4/stream	0.002540	0.05670	**26.7716535**	**0.07407407**	**0.090944882**	**0.16141732**	157.480315	2998.23633	787.401575	330.708661
D5/pond	0.05880	0.1720	**1.15646259**	**0.0244186**	**0.003928571**	**0.00697279**	**6.80272109**	988.372093	34.0136054	14.2857143
D5/stream	0.002690	0.05880	**25.2788104**	**0.07142857**	**0.085873606**	**0.15241636**	148.698885	2891.15646	743.494424	312.267658
D6/ditch	0.06410	0.3260	**1.06084243**	**0.01288344**	**0.003603744**	**0.00639626**	**6.24024961**	521.472393	31.201248	13.1045242
R1/pond	0.04140	0.580	**1.64251208**	**0.00724138**	**0.00557971**	**0.00990338**	**9.66183575**	293.103448	48.3091787	20.2898551
R1/stream	0.002840	0.07970	**23.943662**	**0.05269762**	**0.081338028**	**0.1443662**	140.84507	2132.99875	704.225352	295.774648
R3/stream	0.05830	5.1970	**1.16638079**	**0.00080816**	**0.003962264**	**0.00703259**	**6.86106346**	32.7111795	34.3053173	14.4082333
R4/stream	0.04170	0.5150	**1.63069544**	**0.00815534**	**0.005539568**	**0.00983213**	9.59232614	330.097087	47.9616307	20.1438849
TER criterion			100	10	100	10	10	10	10	10

Table B.9.4-8: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in potatos]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	***Oncorhynchus mykiss***	***Hyalella azteca***	***Americamysis bahia***	***Chironomus riparius***	***Chironomus riparius***	***Pseudokirchn. subcapitata***	***Lemna gibba***
			LC_{50} (µg/L)	**NOEC (µg/L)**	**EC_{50} (µg/L)**	**NOEC (µg/L)**	**NOEC (µg/L)**	**EC_{10} (µg/Kg)**	**E_bC_{50} (µg/L)***	**E_rC_{50} (µg/L)**
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D3/ditch	0.03140	0.1220	**2.1656051**	**0.03442623**	**0.00735669**	**0.01305732**	12.7388535	1393.44262	63.6942675	26.7515924
D4/pond	0.00141	0.0280	**48.2269504**	**0.15**	**0.16382979**	**0.29078014**	283.687943	6071.42857	1418.43972	595.744681
D4/stream	0.02640	0.03230	**2.57575758**	**0.13003096**	**0.00875**	**0.0155303**	15.1515152	5263.15789	75.7575758	31.8181818
D6/ditch	0.03110	0.09160	**2.18649518**	**0.04585153**	**0.00742765**	**0.01318328**	12.8617363	1855.8952	64.3086817	27.0096463
D6/ditch	0.03120	0.09930	**2.17948718**	**0.04229607**	**0.00740385**	**0.01314103**	12.8205128	1711.98389	64.1025641	26.9230769
R1/pond	0.00141	0.03740	**48.2269504**	**0.11229947**	**0.16382979**	**0.29078014**	283.687943	4545.45455	1418.43972	595.744681
R1/stream	0.02160	0.5150	**3.14814815**	**0.00815534**	**0.01069444**	**0.01898148**	18.5185185	330.097087	92.5925926	38.8888889
R2/stream	0.02850	0.5000	**2.38596491**	**0.0084**	**0.00810526**	**0.01438596**	14.0350877	340	70.1754386	29.4736842
R3/stream	0.03040	0.2530	**2.23684211**	**0.01660079**	**0.00759868**	**0.01348684**	13.1578947	671.936759	65.7894737	27.6315789
TER criterion			100	10	100	10	10	10	10	-

Table B.9.4-9: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in potatos]

Scenario	PEC global max (µg/L)	PECsediment global max (µg/kg)	Fish acute	Fish ELS	Invertebrates acute	Invertebrates prolonged	Sed. dweller prolonged	Sed. dweller prolonged	Algae	Aquat. plant
			Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Hyalella azteca*	*Americamysis bahia*	*Chironomus riparius*	*Chironomus riparius*	*Pseudokirchn. subcapitata*	*Lemna gibba*
			LC_{50} (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	NOEC (µg/L)	NOEC (µg/L)	EC_{10} (µg/Kg)	E_bC_{50} (µg/L)*	E_rC_{50} (µg/L)
			0.068	0.0042	0.000231	0.00041	0.4	170	> 2	> 0.84
FOCUS Step 3										
D3/ditch	0.05230	0.2040	**1.3001912**	**0.02058824**	**0.00441683**	**0.00783939**	**7.64818356**	833.333333	38.2409178	16.0611855
D4/pond	0.002360	0.04660	**28.8135593**	**0.09012876**	**0.09788136**	**0.17372881**	169.491525	3648.06867	847.457627	355.932203
D4/stream	0.0440	0.05380	**1.54545455**	**0.07806691**	**0.00525**	**0.00931818**	**9.09090909**	3159.8513	45.4545455	19.0909091
D6/ditch	0.05180	0.1530	**1.31274131**	**0.02745098**	**0.00445946**	**0.00791506**	**7.72200772**	1111.11111	38.6100386	16.2162162
D6/ditch	0.05190	0.1660	**1.31021195**	**0.0253012**	**0.00445087**	**0.00789981**	**7.70712909**	1024.09639	38.5356455	16.1849711
R1/pond	0.002360	0.06240	**28.8135593**	**0.06730769**	**0.09788136**	**0.17372881**	169.491525	2724.35897	847.457627	355.932203
R1/stream	0.0360	0.8590	**1.88888889**	**0.00488941**	**0.00641667**	**0.01138889**	11.1111111	197.90454	55.5555556	23.3333333
R2/stream	0.04750	0.8340	**1.43157895**	**0.00503597**	**0.00486316**	**0.00863158**	**8.42105263**	203.83693	42.1052632	17.6842105
R3/stream	0.05070	0.4220	**1.34122288**	**0.00995261**	**0.00455621**	**0.00808679**	**7.88954635**	402.843602	39.4477318	16.5680473
TER criterion			100	10	100	10	10	10	10	-

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 1, 2, 3), the calculated TER values for the acute and long-term risk resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP (field uses) of the formulation Bulldock 25 EC do not achieve the acceptability criteria TER ≥ 100 and TER ≥ 10 for fish and aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term and acute effects.

Therefore a refined higher tier risk assessment is conducted (please refer to B.9.4.3)

B.9.4.3 Refined risk assessment

B.9.4.3.1 Refined risk assessment based on FOCUS STEP 4 calculations

Table B.9.4-10: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in winter wheat, application in spring] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 % drift reduction	Fish acute	Fish chronic	Invertebrates
		SSD	*Oncorhynchus mykiss*	*Overall assessment/microcosm*
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC (µg/L)
	PEC (µg/L)	**0.312**	**0.0042**	**0.000105**
D1/ditch	0.0003010	1036.54485	13.95348837	**0.348837209**
D1/stream	0.00030	1040	14	**0.35**
D2/ditch	0.0003070	1016.28664	13.68078176	**0.342019544**
D2/stream	0.0003060	1019.60784	13.7254902	**0.343137255**
D3/ditch	0.0003030	1029.70297	13.86138614	**0.346534653**
D4/pond	0.0001140	2736.84211	36.84210526	**0.921052632**
D4/stream	0.0002690	1159.8513	15.6133829	**0.390334572**
D5/pond	0.0001250	2496	33.6	**0.84**
D5/stream	0.0002980	1046.97987	14.09395973	**0.352348993**
D6/ditch	0.0003050	1022.95082	13.7704918	**0.344262295**
R1/pond	0.0002240	1392.85714	18.75	**0.46875**
R1/stream	0.000110	2836.36364	38.18181818	**0.954545455**
R3/stream	0.0003170	984.227129	13.24921136	**0.331230284**
R4/stream	0.0002830	1102.4735	14.8409894	**0.371024735**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjustted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label.

Table B.9.4-11: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in winter wheat, application in autumn] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 % drift reduction	Fish acute	Fish chronic	Invertebrates
		SSD	*Oncorhynchus mykiss*	*Overall assessment/microcosm*
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC (µg/L)
	PEC (µg/L)	0.312	0.0042	0.000105
D1/ditch	0.0003010	1036.54485	13.9534884	**0.34883721**
D1/stream	0.0003420	912.280702	12.2807018	**0.30701754**
D2/ditch	0.0003060	1019.60784	13.7254902	**0.34313725**
D2/stream	0.0002950	1057.62712	14.2372881	**0.3559322**
D3/ditch	0.0003020	1033.11258	13.9072848	**0.34768212**
D4/pond	0.0002940	1061.22449	14.2857143	**0.35714286**
D4/stream	0.0001130	2761.06195	37.1681416	**0.92920354**
D5/pond	0.0003170	984.227129	13.2492114	**0.33123028**
D5/stream	0.0001200	2600	35	**0.875**
D6/ditch	0.0003050	1022.95082	13.7704918	**0.3442623**
R1/pond	0.0003360	928.571429	12.5	**0.3125**
R1/stream	0.0001270	2456.69291	33.0708661	**0.82677165**
R3/stream	0.0003600	866.666667	11.6666667	**0.29166667**
R4/stream	0.0004890	638.03681	8.58895706	**0.21472393**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjustted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label.

Table B.9.4-12: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in spring wheat, application in spring] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 % drift reduction	Fish acute	Fish chronic	Invertebrates
		SSD	*Oncorhynchus mykiss*	*Overall assessment/microcosm*
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC(µg/L)
	PEC (µg/L)	0.312	0.0042	0.000105
D1/ditch	0.000309	1009.70874	13.59223301	**0.33980583**
D1/stream	0.000301	1036.54485	13.95348837	**0.34883721**
D2/ditch	0.000303	1029.70297	13.86138614	**0.34653465**
D2/stream	0.000111	2810.81081	37.83783784	**0.94594595**
D3/ditch	0.000281	1110.32028	14.94661922	**0.37366548**
D4/pond	0.0001270	2456.69291	33.07086614	**0.82677165**
D4/stream	0.000291	1072.16495	14.43298969	**0.36082474**
D5/pond	0.0002820	1106.38298	14.89361702	**0.37234043**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjustted tier-2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label.

Table B.9.4-13: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in winter wheat, application in spring] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 %	Fish acute	Fish chronic	Invertebrates
		SSD	*Oncorhynchus mykiss*	*Overall assessment/microcosm*
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC (µg/L)
	PEC (µg/L)	0.312	0.0042	0.000105
D1/ditch	0.0006010	519.134775	**6.988352745**	**0.174708819**
D1/stream	0.000330	945.454545	12.72727273	**0.318181818**
D2/ditch	0.0003070	1016.28664	13.68078176	**0.342019544**
D2/stream	0.0006120	509.803922	**6.862745098**	**0.171568627**
D3/ditch	0.0003030	1029.70297	13.86138614	**0.346534653**
D4/pond	0.0001140	2736.84211	36.84210526	**0.921052632**
D4/stream	0.0005390	578.849722	**7.792207792**	**0.194805195**
D5/pond	0.0001250	2496	33.6	**0.84**
D5/stream	0.0005960	523.489933	**7.046979866**	**0.176174497**
D6/ditch	0.0003050	1022.95082	13.7704918	**0.344262295**
R1/pond	0.0004490	694.877506	**9.354120267**	**0.233853007**
R1/stream	0.000110	2836.36364	38.18181818	**0.954545455**
R3/stream	0.0006330	492.890995	**6.63507109**	**0.165876777**
R4/stream	0.0004710	662.420382	**8.917197452**	**0.222929936**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjusted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label.

Table B.9.4-14: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in winter wheat, application in autumn] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 %	Fish acute	Fish chronic	Invertebrates
		SSD	*Oncorhynchus mykiss*	*Overall assessment/microcosm*
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC (µg/L)
	PEC (µg/L)	0.312	0.0042	0.000105
D1/ditch	0.0006020	518.272425	**6.976744186**	**0.1744186**
D1/stream	0.0003420	912.280702	12.28070175	**0.30701754**
D2/ditch	0.000306	1019.60784	13.7254902	**0.34313725**
D2/stream	0.0005910	527.918782	**7.106598985**	**0.17766497**
D3/ditch	0.0003020	1033.11258	13.90728477	**0.34768212**
D4/pond	0.0005870	531.516184	**7.155025554**	**0.17887564**
D4/stream	0.0001130	2761.06195	37.16814159	**0.92920354**
D5/pond	0.0006340	492.113565	**6.624605678**	**0.16561514**
D5/stream	0.0001200	2600	35	**0.875**
D6/ditch	0.0003050	1022.95082	13.7704918	**0.3442623**
R1/pond	0.0005610	556.149733	**7.486631016**	**0.18716578**
R1/stream	0.0001280	2437.5	32.8125	**0.8203125**
R3/stream	0.000638	489.028213	**6.5830721**	**0.1645768**
R4/stream	0.0008140	383.292383	**5.15970516**	**0.12899263**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjusted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label

Table B.9.4-15: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in spring wheat, application in spring] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 % drift reduction	Fish acute	Fish chronic	Invertebrates
		SSD	***Oncorhynchus mykiss***	***Overall assessment/microcosm***
		median HC5 LC_{50} (µg/L)	**NOEC (µg/L)**	**RAC (µg/L)**
	PEC (µg/L)	0.312	0.0042	**0.000105**
D1/ditch	0.000309	1009.70874	13.59223301	**0.33980583**
D1/stream	0.000601	519.134775	**6.988352745**	**0.17470882**
D3/ditch	0.000303	1029.70297	13.86138614	**0.34653465**
D4/pond	0.000111	2810.81081	37.83783784	**0.94594595**
D4/stream	0.000563	554.174067	**7.460035524**	**0.18650089**
D5/pond	0.000127	2456.69291	33.07086614	**0.82677165**
D5/stream	0.000582	536.082474	**7.216494845**	**0.18041237**
R4/stream	0.000470	663.829787	**8.936170213**	**0.22340426**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjustted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in spring/winter wheat according to the label.

Table B.9.4-16: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 7.5 g as/ha (14 d) in potatoes] (FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 %	Fish acute	Fish chronic	Invertebrates
		SSD	***Oncorhynchus mykiss***	***Overall assessment/microcosm***
		median HC5 LC_{50} (µg/L)	**NOEC (µg/L)**	**RAC (µg/L)**
	PEC (µg/L)	0.312	0.0042	0.000105
D3/ditch	0.0003020	1033.11258	13.9072848	**0.34768212**
D4/pond	0.0001090	2862.38532	38.5321101	**0.96330275**
D4/stream	0.0002750	1134.54545	15.2727273	**0.38181818**
D6/ditch	0.0002990	1043.47826	14.0468227	**0.35117057**
D6/ditch	0.0003000	1040	14	**0.35**
R1/pond	0.0001120	2785.71429	37.5	**0.9375**
R1/stream	0.0002250	1386.66667	18.6666667	**0.46666667**
R2/stream	0.0002970	1050.50505	14.1414141	**0.35353535**
R3/stream	0.0003180	981.132075	13.2075472	**0.33018868**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjusted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in potatos according to the label.

Table B.9.4-17: Maximum PEC_{SW} values and TER values for beta-cyfluthrin [2 x 12.5 g as/ha (14 d) in potatoes](FOCUS STEP 4 -20 m + 90 % drift reduction measures)

Focus scenario	Step 4 20m + 90 %	Fish acute	Fish chronic	Invertebrates
		SSD	Oncorhynchus mykiss	Overall assessment/microcosm
		median HC5 LC_{50} (µg/L)	NOEC (µg/L)	RAC (µg/L)
	PEC (µg/L)	0.312	0.0042	0.000105
D3/ditch	0.0003020	1033.11258	13.9072848	**0.34768212**
D4/pond	0.0001090	2862.38532	38.5321101	**0.96330275**
D4/stream	0.0005490	568.306011	**7.65027322**	**0.19125683**
D6/ditch	0.0002990	1043.47826	14.0468227	**0.35117057**
D6/ditch	0.00030	1040	14	**0.35**
R1/pond	0.0001150	2713.04348	36.5217391	**0.91304348**
R1/stream	0.0004490	694.877506	**9.35412027**	**0.23385301**
R2/stream	0.0005930	526.13828	**7.08263069**	**0.17706577**
R3/stream	0.0006360	490.566038	**6.60377358**	**0.16509434**
TER criterion		9	10	1

Based on the calculated concentrations of beta-cyfluthrin in surface water (PEC_{SW} FOCUS Step 4 – 20 m + 90 % drift reduction), the calculated TER values for the acute and long-term risk to fish resulting from an exposure of aquatic organisms to beta-cyfluthrin according to the GAP of the formulation Bulldock EC 25 achieve the adjusted tier -2 acceptability criterion TER ≥ 9 for fish (acute) and acceptability criterion TER ≥ 10 for fish (chronic) , but do not achieve tier – 3 RAC for aquatic invertebrates, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for long-term effects.

The results of the assessment indicate an unacceptable risk for aquatic organisms due to the intended (representative) use of Bulldock EC 25 in potatos according to the label.

B.9.4.3.2 Refined risk assessments based on roughly estimates of further refinements proposed by the notifier (Ranke, J. 2014)

In the amendment by Ranke 2014 further drift and run-off mitigation measures are calculated in FOCUS Step 4: drift buffer zone of 50 m and 60 m, nozzle reduction of up to 95 %, 10 m vegetated filter strip and a combination of drift buffer zone plus nozzle reduction plus vegetated filter strip. As a result, a strong reduction of PECsw (PECsw –Step 3 to PECsw-Step 4) could be achieved (up to 97.8 %). When appling this high reduction to the lowest PECsw-Step 3 (0.0014 µg/L in D4-stream and R1-pond, application of 2 x 7.5 g as/ha in potatoes) an acceptable concentration in water (0.00003102 µg/L) can be achieved.

Conclusion:
Based on roughly estimates considering further refinements proposed by Ranke, J. (2014, please refer also to Volume_3CP_Bulldock EC 25_B-8.5) a safe use of Bulldock EC 25 as a spray application in

wheat and was demonstrated for one Scenario (R1).
However, the proposed risk mitigation measures are indeed theoretically possible, but not practicable in all EU Member States (e.g. in Germany)

B.9.5 Effects on arthropods

B.9.5.1 Effects on bees (KCP 10.3.1)

Bulldock 25 EC is an emulsifiable concentrate supported for renewing the approval of beta-cyfluthrin containing 25 g beta-cyfluthrin/L. Effects of Bulldock 25 EC on bees were evaluated as part of the first EU review of the active substances beta-cyfluthrin. However in the meantime new studies have been performed. Therefore all relevant data and assessments are provided here and are considered adequate.

B.9.5.1.1 Acute toxicity (KCP 10.3.1.1)

Acute oral (KCA 10.3.1.1.1) and contact (KCA 10.3.1.1.2) toxicity

Report:	CP 9.5.1.1/1 Pinsdorf W., 1987 Ergebnis der Zulassungsprüfung auf Bienengefährlichkeit - Versuchsplan 1987- Firmenauftrag (Laboratoriumsprüfung) Landwirtschaftskammer Münster, Germany, report no.: B-87281
Guidelines:	BBA Guideline VI, 23-1
GLP:	no

Executive Summary

The toxicity of Bulldock 25 EC to honeybees was determined in laboratory tests. In feeding tests the LD_{50} was < 0.025 µg as/bee.

RMS's comments:
This study is considered valid and in principle acceptable for the risk assessment. However, since the experimental conditions do not fulfill present-day requirements (e.g. GLP condition, tests according to EPPO Guideline 170 or OECD Guideline 213/214) the provided information will only be used as additional information.

Report:	CP 9.5.1.1/2 Stute K., 1987 Ergebnis der Zulassungsprüfung auf Bienengefährlichkeit 1987 – Laboratoriumsprüfung Landesinstitut für Bienenkunde Celle, Germany, report no.: B-87280
Guidelines:	BBA Guideline VI, 23-1
GLP:	no

Executive Summary

The toxicity of Bulldock 25 EC to honeybees was determined in laboratory tests. In feeding tests the LD_{50} of Bulldock 25 EC to honeybees was < 0.25 µg as/bee.

RMS's comments:
This study is considered valid and in principle acceptable for the risk assessment. However, since the experimental conditions do not fulfill present-day requirements (e.g. GLP condition, tests according to EPPO Guideline 170 or OECD Guideline 213/214) the provided information will only be used as additional information.

Report:	CP 9.5.1.1/3 Mautz, 1987 Prüfung auf Bienengefährlichkeit für das Zulassungs-verfahren – Laboratoriumsprüfung Bayerische Landesanstalt für Bienenzucht, Erlangen, Germany, report no.: B-870185
Guidelines:	BBA Guideline VI, 23-1
GLP:	no

Executive Summary

The toxicity of Bulldock 25 EC to honeybees was determined in laboratory tests. In feeding tests the LD_{50} of Bulldock 25 EC to honeybees was < 0.25 µg as/bee.

RMS's comments:
This study is considered valid and in principle acceptable for the risk assessment. However, since the experimental conditions do not fulfill present-day requirements (e.g. GLP condition, tests according to EPPO Guideline 170 or OECD Guideline 213/214) the provided information will only be used as additional information.

Report:	CP 9.5.1.1/4 Schmitzer, S., Sekine, T. 2010 Effects of beta-cyfluthrin EC 025 G (acute contact and oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory, IBACON, Rossdorf, Germany, report no.: 52601035
Guidelines:	OECD 2013 and OECD 214 (1998)
GLP:	yes

Executive Summary

In an acute laboratory study the contact and oral toxicity of beta-cyfluthrin EC 025 G to the honey bee, *Apis mellifera* L., were tested.
Female worker bees were exposed to nominal 3.1, 6.3, 12.5, 25.0, 50.0 and 100 ng as/bee for contact and oral toxicity.
The contact test was prolonged to 96 h, since mortality between 24 and 48 h and between 48 and 72 h was increasing.
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for oral toxicity and 4, 24, 48, 72 and 96 h after test initiation for contact toxicity.
Mortality was observed in the contact toxicity test in the three highest concentrations (25.0, 50.0 and 100 ng as/bee) and in the control and 3.1 ng as/bee concentration at 96 h (< 10 %). In the oral toxicity test mortality was observed at all test concentration except the lowest (3.1 ng as/bee).
All validity criteria according to OECD 213 and OECD 214 were fulfilled.

The LD_{50} (96 h) was 0.0337 µg as/bee in the contact test and 0.0164 µg as/bee in the oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	beta-cyfluthrin EC 025 G
Description:	Colourless liquid
Lot/Batch #:	PF90225222
Purity:	2.93 % (26.11 g/L)

2. Vehicle and/or positive control: Control contact test: Water + 0.5 % Adhäsit
Control oral test: 50 % aqueous sugar solution in tap water
Positive control: Perfekthion (BAS 152 11I): 400g/L dimethoate

3. Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Own colony
Diet/Food:	Commercial ready-to-use syrup (Apiinvert: 30 % saccharose, 31 % glucose, 39 % fructose)

4. Environmental conditions:

Temperature:	25 °C
Humidity:	55 - 85 %
Photoperiod:	24 hours darkness (except during observation)

B. STUDY DESIGN AND METHODS

1. Experimental treatments
Contact test: The definitive test was conducted with 3.1, 6.3, 12.5, 25.0, 50.0 and 100.0 ng as/bee prepared in an appropriate carrier (tap water with 0.5 % adhäsit) and administered after anesthetisation as a 5 µL droplet per bee (dorsal thorax) to each of ten bees in each of three cages per treatment. A control with 0.5 % Adhäsit in tap water for contact toxicity as well as a toxic reference (400 g/L dimethoate) were run in parallel. During the observation method a 50 % aqueous sugar solution was provided.

Oral test: The definitive test was conducted with 3.1, 6.3, 12.5, 25.0, 50.0 and 100.0 ng as/bee in treated sugar solution (50 % aqueous sugar solution in tap water), offered over a period of 6 hours. A control of 0.5 % aqueous sugar solution and the toxic reference (400 g/L dimethoate) were prepared analogically.
The treated food was offered in syringes, which were weighed before and after introduction into the cages. Duration of uptake was 6 hours for the test item treatments. At the highest treatment level the mean dose consumed was 43.2 µg as/bee.

2. Observations
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for oral toxicity and after 4, 24, 48, 72 and 96 h for contact toxicity.

3. Statistical calculations
The contact and oral LD_{50} of the test item was estimated with Probit analysis and for the reference item according to moving average computations. The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-1: Toxicity of beta-cyfluthrin EC 025 G to honey bees (*Apis mellifera*) in the acute tests

Dose [ng as/bee]	Mean intake [ng as/bee]	Mortality [%]				
		4 h	24 h	48 h	72 h	96 h
contact toxicity test						
control	-	0	0	0	0	6.7
3.1	-	0	0	0	0	6.7
6.3	-	0 [a]	0	0	0	0
12.5	-	0 [a]	0	0	0	0
25.0	-	0 [a]	23.3 [a]	30.0 [a]	30.0 [a]	30.0 [a]
50.0	-	0 [a]	66.7 [a]	83.3 [a]	83.3 [a]	83.3 [a]
100.0	-	0 [a]	50.0 [a]	83.3 [a]	100.0 [a]	100.0 [a]
oral toxicity test						
control		0	0	0	-	-
3.1	3.5	0	0	0	-	-
6.3	6.8	3.3 [a]	10.0	10.0	-	-
12.5	11.9	0 [a]	36.7	40.0	-	-
25.0	20.0	13.3 [a]	63.3	63.3	-	-
50.0	41.5	30.0 [a]	93.3 [a]	96.7	-	-
100.0	43.2	30.0 [a]	76.7	80.0	-	-

[a] behavioural abnormalities observed

B. OBSERVATIONS

In both test setups, mortality was < 10 % for the control and the lowest test concentration. In the oral toxicity test the maximum nominal test level of 100.0 ng test item/bee corresponded to an actual intake of 43.2 ng as/bee. Sublethal effects were observed for most concentrations in the beginning of the tests. For contact toxicity mortality > 10 % was observed at the three highest test concentrations (25.0, 50.0 and 100.0 ng as/bee) and for oral toxicity for all concentrations except 3.1 ng as/bee.

All validity criteria according to OECD 213 and OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10 % and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSION

The toxicity of beta-cyfluthrin EC 025 G was tested in an acute contact and an oral toxicity test on honey bees. The LD_{50} (96 h) was 33.7 ng as/bee (0.0337 µg as/bee) in the contact toxicity test, in the oral toxicity test (48 h) it was 16.4 ng as/bee (0.0164 µg as/bee).

RMS's comments:

This study is considered valid and acceptable for the risk assessment.

B.9.5.1.2 Chronic toxicity (KCP 10.3.1.2)

The chronic toxicity of Bulldock 25 EC to adult honey bees was assessed in a 10-day chronic feeding test. A summary of the study is provided below.

Report: CP 9.5.1.2/1
Sandrock, C. 2014a
Bulldock 25 EC: Toxicity effects to honey bee (*Apis mellifera* L.) worker adults after oral chronic exposure under laboratory conditions,
Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland, report no.: 20120186

Guidelines: CEB Method No. 230. Method for the evaluation of the impact of plant Protection products on honey bees. 2003.
Decourtye *et al*. 2005. Comparative sub-lethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch. Environ. Contam. Toxicol. 48, 242-250.
Suchail *et al*. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ. Toxicol. Chem. 20, 2482-2486.

GLP: yes

Executive Summary

Two-day-old worker bees were fed *ad libitum* with a 50 % (w/v) sucrose solution incorporating the test treatment (Bulldock 25 EC) at appropriate concentrations for 10 consecutive days. Four replicate units, each consisting of 30 worker bees, were established for each treatment. Mortality, behavioural abnormalities and food consumption were determined once a day over a period of 10 days of chronic oral exposure.

Treatments:
1 control
2 reference item concentrations (target doses: 0.0232, 0.0116 µg dimethoate/bee/day)
5 test item concentrations (target doses: 0.08, 0.04, 0.02, 0.01, 0.005 µg as/bee/day; effective doses: 0.068, 0.041, 0.012, 0.007, 0.003 µg as/bee/day)

After 10 days of chronic oral exposure mortality of honeybees in the control treatment was 2.5 %. Mortality of 100, 85.8, 24.2, 11.7 and 1.7 % was observed in the 0.068, 0.041, 0.012, 0.007 and 0.003 µg as/bee/day, respectively. Mortality in the reference item treatments was 100.0 and 45.0 % at 0.020 and 0.007 µg as/bee/day, respectively, demonstrating the sensitivity of the test system.
No statistically significant differences were observed with the control treatment and the two lowest doses tested.

On day 1 and 2, signs of aggressiveness were observed in all replicates of test item treatment 0.012 and 0.041 µg as/bee/day as well as in all replicates of both reference item doses. Symptoms of poisoning were observed for the test item treatments 0.007 µg as/bee/day (day 4 and 6, 1 replicate each), 0.041 µg as/bee/day (day 4, 3 replicates; day 5, 2 replicates and day 6, 1 replicate) and 0.068 µg as/bee/day (day 4, 3 replicates and day 5, 2 replicates).

All validity criteria were met and, therefore, the study can be considered as valid.

In conclusion, after 10 days of oral chronic exposure of honeybee worker adults to beta-cyfluthrin, cumulative mortality was not statistically significant different between the control and the two lowest test item doses tested.
LD_{50} values were determined to be 0.019 µg as/bee/day.
The NOAED value for beta-cyfluthrin was determined to be 0.007 µg as/bee/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC (active ingredient: beta-cyfluthrin)
Description:	liquid
Lot/Batch #:	92110454
Purity:	25.8 g/L
Density:	0.901 g/mL
2. Vehicle and/or positive control:	Control: 50 % (w/v) sucrose solution Positive control: Dimethoate 99.5 %

3. Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult newly emerged worker bees (2 days old)
Source:	The test organisms were obtained from honeybee colonies provided by a commercial beekeeper (Jacques Breiter, Sissach, Switzerland).
Diet/Food:	During the first two days after emergence, worker bees from all treatments were fed ad libitum with 50 % (w/v) aqueous sucrose solution and a paste made of powder sugar, pollen and water (11.9 g : 12.5 g : 0.9 g).

4. Environmental conditions:

Temperature:	28 ± 2 °C
Humidity:	60 ± 15 %
Photoperiod:	24 hours darkness

B. STUDY DESIGN AND METHODS

1. Experimental treatments:
The study consisted of eight treatments: a control, five test item concentrations and two reference item concentrations. Four replicate units, each consisting of 30 worker bees, were established for each treatment. Therefore, a total of 120 bees were set up for each treatment.

The target test item doses for the definitive test chosen were as follows: 0.08, 0.04, 0.02, 0.01 and 0.005 µg as/bee/day.
The target reference item doses were 0.0232 and 0.0116 µg dimethoate/bee/day.
The control treatment consisted of 50 % (w/v) sucrose solution.

Oral exposure started three days after bee emergence. Worker bees were fed *ad libitum* for 10 consecutive days with 50 % (w/v) sucrose solution only or solutions incorporating the test or reference items at different concentrations. A volume of 50 µL solution per bee in a 2 mL vial was weighed and placed in each test unit. Every day vials were removed from the test units and weighed to determine the amount of solution consumed. Fresh test solutions were supplied daily.

2. Observations:
Mortality was assessed once a day. Bees were noted as moribund when they were on their back or side, still twitching, but unable to right themselves. Bees were noted as dead when no reaction to a tactile stimulation was observed. Dead bees were removed from the cages. Behavioral abnormalities,

e.g., uncoordinated movement, trembling, tumbling, hypo/hyperresponsiveness and hypo/hyperactivity, abnormal movements of legs or wings, etc., were also recorded once a day to assess sub-lethal effects of the test item.
The consumption of the sucrose solution was determined by recording the weight of the vials before and after treatment. The dose consumed per bee was calculated by dividing the amount of sucrose solution consumed by the number of healthy, surviving bees.

3. Statistical calculations:
For data analysis, results of the different test item treatments were compared with the control. Cumulative mortality after 10 days of exposure was analysed using Analysis of Variance (ANOVA). The level of significance was $\alpha = 0.05$. Student's t-tests with Bonferroni-Holm adjustments of significance levels were used for comparisons of the control mean mortality rate with those of various test item treatments (test direction: one-sided, greater). The estimate of the NOAED was based on the highest test item dose that did not cause significantly increased mortality compared to the control. LD_{10}, LD_{20} and LD_{50} values with 95 % confidence limits were calculated using Probit analysis. For all tests, $\alpha = 0.05$.
Statistical analysis was performed using ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION
A. FINDINGS

Table B.9.5-2: Toxicity of Bulldock 25 EC to honey worker bees (*Apis mellifera*) after Oral Chronic Exposure

	Effective dose tested (µg as/bee/day)					
	Control	**0.003**	**0.007**	**0.012**	**0.041**	**0.068**
Cumulative mortality [%]	2.5	1.7	11.7	24.2*	85.8*	100.0*
NOAED	0.007 µg as/bee/day					
LD_{50} (95 %-CI) [1]	0.019 (0.016 – 0.023) µg as/bee/day					
* Statistically significant differences when compared to the control (Student's t-tests with Bonferroni-Holm adjustment; $\alpha = 0.05$) [1] Calculated with Probit analysis CI = confidence interval						

B. OBSERVATIONS

Consumption of sugar solution in the control treatment during the definitive test was 21.38 µl/bee/day. In the test item treatments, consumption ranged between 12.68 and 21.43 µl/bee/day. Interval consumption of sugar solution in the reference item treatments ranged from 11.84 and 17.34 µl/bee/day. After 10 days of chronic oral exposure mortality of honeybees in the control treatment was 2.5 %.
Mortality of 100, 85.8, 24.2, 11.7 and 1.7 % was observed in the 0.068, 0.041, 0.012, 0.007 and 0.003 µg as/bee/day, respectively. Mortality in the reference item treatments was 100.0 and 45.0 % at 0.020 and 0.007 µg as/bee/day, respectively, demonstrating the sensitivity of the test system.
No statistically significant differences were observed with the control treatment and the two lowest doses tested.

On day 1 and 2, signs of aggressiveness were observed in all replicates of test item treatment 0.012 and 0.041 µg as/bee/day as well as in all replicates of both reference item doses. Symptoms of poisoning were observed for the test item treatments 0.007 µg as/bee/day (day 4 and 6, 1 replicate

each), 0.041 µg as/bee/day (day 4, 3 replicates; day 5, 2 replicates and day 6, 1 replicate) and 0.068 µg as/bee/day (day 4, 3 replicates and day 5, 2 replicates).

At study termination, honeybee worker mortality in the control treatment was 2.5 %. Mortality in the reference item treatment was 100.0 and 45.0 % at an effective dose of 0.020 and 0.007 µg dimethoate/bee/day, respectively.
The validity criteria for control (< 15 % mortality), the higher reference item treatment of 0.020 µg dimethoate/bee/day (target 0.0232 µg dimethoate/bee/day; > 50 % mortality) and the lower reference item treatment of 0.007 µg dimethoate/bee/day (target 0.0116 µg dimethoate/bee/day; approximately 50 % mortality) were met.

Therefore all validity criteria were met and, the study can be considered as valid.

III. CONCLUSION

In conclusion, after 10 days of oral chronic exposure of honeybee worker adults to beta-cyfluthrin, cumulative mortality was not statistically significant different between the control and the two lowest test item doses tested.
LD_{50} values were determined to be 0.019 µg as/bee/day.
The NOAED value for beta-cyfluthrin was determined to be 0.007 µg as/bee/day.

RMS's comments:
This study is considered valid and acceptable for the risk assessment.

B.9.5.1.3 Effects on honeybee brood (KCP 10.3.1.3)

The acute toxicity of Bulldock 25 EC to bee larvae was assessed in a laboratory study. A summary of the study is provided below.

Report:	CP 9.5.1.2/2 Sandrock, C. 2014b Bulldock 25 EC: Toxicity Effects to Honey Bee (Apis mellifera L.) Larvae after Single Exposure under Laboratory Conditions Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland report no.: 20120187
Guidelines:	OECD Draft Guideline 2012. Honey bee (*Apis mellifera*) larval toxicity test, single exposure.
GLP:	yes

Executive Summary

The purpose of the study was to determine the acute toxicity effects of Bulldock 25 EC (as beta-cyfluthrin) to honeybee larvae (*Apis mellifera* L.) under laboratory conditions.
Synchronised 4-day-old honeybee larvae were fed with five test item concentrations incorporated into the larval diet. The effective doses were 0.0066, 0.0133, 0.0277, 0.0491 and 0.1116 µg as/larva.

Three replicates per treatment were used. Each replicate consisted of twelve larvae. Larvae used in the study originated from six different colonies. Seven 48-well tissue cellular culture plates, each containing at least 36 larvae, were used in the study: one for the control, one for the reference item, and five plates for the test item treatment. For control untreated diet was used, Dimethoate (8.8 µg as per larva) was used as reference item.
Following the administration of the test item on day 4 (D4), mortality was recorded on day 5 (D5), day 6 (D6) and at termination on day 7 (D7) (i.e., 24 h, 48 h and 72 h after administration of test item, respectively).

Effects caused by the test item 72-hours after single feeding were compared with the untreated control. LD_{10}, LD_{20} and LD_{50}, and NOED values were calculated.

At study termination, control mortality was 0.0 %. At test termination (D7), corresponding to 72-hours after single exposure, the cumulative mortality was 0.0, 30.6, 72.2, 91.7 and 97.2 for the effective doses of 0.0066, 0.0133, 0.0277, 0.0491 and 0.1116 µg as/larva, respectively. Except for the lowest effective dose (0.0066 µg as/larva), all other test item treatments (i.e. 0.0133, 0.0277, 0.0491 and 0.1116 µg as/larva) exhibited significantly increased mortality compared to the control.
Mortality in the reference item treatment was 80.6 %, demonstrating the sensitivity of the test system.
All validity criteria were met and, therefore, the study can be considered as valid.

The LD_{50} values 72-hours after single exposure of honeybee larvae to beta-cyfluthrin (the active substance of Bulldock 25 EC) were determined to be 0.020 µg as/larva, respectively.
The NOED was determined to be 0.007 µg as/larva.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC (active ingredient: beta-cyfluthrin)
Description:	liquid
Lot/Batch #:	92110454
Purity:	25.8 g/L
Density:	0.901 g/mL

2. Vehicle and/or positive control: Control: untreated diet
Positive control: Dimethoate 99.5 %

3. Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	First instar honeybee larvae (4 days old)
Source:	The test organisms were reared at IES Ltd and taken from healthy, disease-free and queen-right colonies
Diet/Food:	Diet A: (D1): 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight fructose. Diet B (D3): 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight of glucose and 15 % weight fructose. Diet C (D4 to D6): 50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of glucose and 18 % weight fructose.

4. Environmental conditions:

Temperature:	Mean 34.7 °C (Range: 32.3 – 35.3 °C)
Relative humidity:	Mean 95.2 % (Range: 68.5 – 101.3 %)
Photoperiod:	24 hours darkness

B: STUDY DESIGN AND METHODS

1. Experimental treatments

The study consisted of seven treatments: control, five test item treatments and a reference item. The experimental unit was the individual grafting cup containing a honeybee larva. Three replicates each were established for the control, the five item treatment concentrations and the reference item. Larvae used in the study originated from three different colonies, with each colony consistently providing larvae for one of the three replicates across treatments. Seven 48-well tissue cellular culture plates, each containing at least 36 larvae, were used in the study: one for the control, one for the reference item, and five plates for the test item treatments. For the definitive test the nominal target test item doses were: 0.0053, 0.0106, 0.0212, 0.0425 and 0.0849 µg as/larva. The control treatment was fed with untreated diet. The reference item treatment dose was 8.8 µg dimethoate per larva.
On D4, 250 µL and 125 µL of test and reference item test solutions, respectively, were mixed with 2500 µL diet solution, thereby adjusting a 10 % v/v ratio to obtain corresponding target doses of the diets for each treatment (density diet = 1.0305 g/mL). Finally, 30 µL test diet, including 3 µL test solution for the test and reference item treatments, was administered to each larva.

2. Observations

Following the administration of the test item on day 4 (D4), mortality was recorded on day 5 (D5), day 6 (D6) and at termination on day 7 (D7) (i.e., 24 h, 48 h and 72 h after administration of test item, respectively). Immobile larvae or larvae which did not react to contact of a paintbrush were noted as dead.
At the study termination (D7) cells in which alive larvae did not entirely consume all provided food (i.e., 'unconsumed food') were recorded, but qualitatively only.
In order to evaluate effective concentrations of the test item solutions A-E, and corresponding effective doses per larvae, two aliquots were taken from the freshly prepared application solution and analysed using GC-MS based on the ionisation modes Electron Impact (EI) and Negative Chemical Ionisation (NCI).

3. Statistical calculations

For data analysis, results of the different test item treatments were compared with the control. Abbott's corrected mortality was not calculated since mortality in the control treatments was 0.0 %. Cumulative larval mortality on D7 of individual test item treatments was analysed in comparison to the control using Fisher's Exact Binomial test with Bonferroni Correction, i.e. significance levels for pair-wise comparisons between treatment and control were adjusted for multiple testing ($\alpha = 0.05$). The LD_{10}, LD_{20} and LD_{50} values with 95 % confidence limits as well as the NOED were calculated for the active ingredient of Bulldock 25 EC. Probit analysis was used to calculate the LDx values. Statistical analysis was performed using ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-3: Effect of Bulldock 25 EC on mortality of honeybee, *Apis mellifera* L., larvae after single exposure on D4

	Control	Bulldock 25 EC (effective dose of as beta-cyfluthrin) (µg as/larva)					Dimethoate (µg as/larva)
		0.0066	0.0133	0.0277	0.0491	0.1116	8.8
Mortality D7 [%]	0.0	0.0	30.6*	72.2*	91.7*	97.2*	80.6
NOED	0.007 µg as/bee/day						
LD_{50} (95 %-CI)	0.020 (0.017 – 0.024) µg as/bee/day						

* Statistically significantly different from the control (Fisher's Exact Binomial test with Bonferroni corrections; $\alpha = 0.05$); CI = confidence interval

B. OBSERVATIONS

The EI (Electron Impact) mean recoveries for beta-cyfluthrin of two individual determinations per dose level were observed to be 145.7 % (target dose level 0.0849 µg as/larva), 156.5 % (target dose level 0.0425 µg as/larva), 144.6 % (target dose level 0.0212 µg as/larva), 139.7 % (target dose level 0.0106 µg as/larva) and 137.6 % (target dose level 0.0053 µg as/larva).
Effective mean recoveries for beta-cyfluthrin, as inferred by NCI (Negative Chemical Ionisation) analyses, were 131.4 % (target dose level 0.0849 µg as/larva), 115.5 % (target dose level 0.0425 µg as/larva), 130.5 % (target dose level 0.0212 µg as/larva), 125.5 % (target dose level 0.0106 µg as/larva) and 123.9 % (target dose level 0.0053 µg as/larva).
Calculations of effective concentrations and doses are based on the more conservative mean recoveries obtained by NCI analyses.

At study termination, cumulative control mortality across replicates was 0.0 % Cumulative mortality in the reference item treatment, dimethoate at 8.8 µg as/larvae, was 80.6 %.
Measured concentrations of the active ingredient were more than 20 % higher than previously defined target concentrations. Therefore, based on residue analyses, all evaluations reported here are conservatively based on re-calculated effective concentrations.
The study can thus be considered as valid.

At study termination, control mortality was 0.0 %.
At test termination (D7), corresponding to 72-hours after single exposure, the cumulative mortality was 0.0, 30.6, 72.2, 91.7 and 97.2 for the effective doses of 0.0066, 0.0133, 0.0277, 0.0491 and 0.1116 µg as/larva, respectively.
Except for the lowest effective dose (0.0066 µg as/larva), all other test item treatments (i.e. 0.0133, 0.0277, 0.0491 and 0.1116 µg as/larva) exhibited significantly increased mortality compared to the control.
In the reference test 8.8 µg as (dimethoate) per larva was dosed. Mortality in the reference item treatment was 80.6 %, demonstrating the sensitivity of the test system.

III. CONCLUSION

The LD_{50} values 72-hours after single exposure of honeybee larvae to beta-cyfluthrin (the active substance of Bulldock 25 EC) were determined to be 0.020 µg as/larva, respectively.
The NOED was determined to be 0.007 µg as/larva.

RMS's comments:

This study is considered valid and acceptable for the risk assessment.

B.9.5.1.4 Sublethal effects (KCP 10.3.1.4)

Sub-lethal effects on honey bees were assessed in cage, tunnel and field tests already available for the EU evaluation of beta-cyfluthrin (2002). Please refer to points B 9.5.1.5 and B 9.5.1.6 below.

In addition two new field studies with Bulldock 25 EC were conducted considering repeated applications. The studies were investigating potential long-term effects on relevant assessment parameters and are summarised under point B 9.5.1.6/1 and B 9.5.1.6/2.

B.9.5.1.5 Cage and tunnel tests (KCP 10.3.1.5)

The effect of Bulldock 25 EC to honey bees under field conditions were evaluated in cage tests conducted according to the BBA-Guideline 23-1 methodology. The data were reviewed in the first Monograph and a summary of the honey bee cage test endpoints is presented in Table B.9.5-4.

Table B.9.5-4: Semi-field tests honey bee endpoints relevant for beta-cyfluthrin

Test substance	Application rate	Exposure	Result	Reference	EU agreed endpoint (SANCO/6841/VI/97-final)
Bulldock 25 EC	15 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - 2 d) reduced. Mortality slightly increased. Colony strength and brood not affected.	Pinsdorf, 1989a Study no.: 890622, 890623	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - 2 d) reduced. Mortality slightly increased. Colony strength and brood not affected.	Pinsdorf, 1989b Study no.: 890624, 890625	Already evaluated
Bulldock 25 EC	37.5 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - 2 d) reduced. Mortality slightly increased.	Stute, 1987a Study no.: 870249	Already evaluated
Bulldock 25 EC	37.5 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - 2 d) reduced. Mortality slightly increased.	Stute, 1987b Study no.: 870248	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - > 3 d) reduced. Mortality slightly to moderately increased.	Stute, 1989a Study no.: 890300 , 890301	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (1 - 3 d) reduced. Mortality not significantly increased. Colony strength and brood not affected.	Schulz, 1989a Study no.: 890901, 890902	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on *Phacelia* (tent study)	Flight intensity transitorily (2 d) reduced. Mortality not or only slightly increased. Colony strength and brood not affected	Stute, 1989b Study no.: 890302, 890303	Already evaluated

B.9.5.1.6 Field tests (KCP 10.3.1.6)

Field studies were conducted to evaluate whether the increased mortality observed in the tent studies was due to starvation associated with the observed repellency effect.

Beta-cyfluthrin 25 EC was applied at 15 g as/ha to flowering *Phacelia* fields in the evening after the bee flight activity or during active bee foraging (7.5 and 15 g as/ha). The data were reviewed in the first Monograph and a summary of the honey bee field test endpoints is presented in (see Table B.9.5-5).

In addition two new field studies with Bulldock 25 EC were conducted considering repeated applications. The studies were investigating potential long-term effects on relevant assessment parameters (refer to point B 9.5.1.6/01 and B 9.5.1.6/02). The results are summarised in Table B.9.5-5.

Table B.9.5-5: Field test honey bee endpoints relevant for beta-cyfluthrin

Test substance	Application rate	Exposure	Result	Reference	EU agreed endpoint (SANCO/6841/VI/97-final)
Bulldock 25 EC	15 g as/ha	Evening application on flowering *Phacelia*	Flight intensity not affected. Mortality not increased. Colony strength and brood not affected.	Stute, 1989c Study no.: 890304	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on flowering *Phacelia*	Flight intensity transitorily reduced. Mortality not increased. Colony strength and brood not affected.	Pinsdorf, 1989a Study no.: 890621	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on flowering *Phacelia*	Flight intensity not affected. Mortality not increased. Colony strength and brood not affected.	Stute, 1989d Study no.: 890305	Already evaluated
Bulldock 25 EC	15 g as/ha	Evening application on flowering *Phacelia*	Flight intensity transitorily reduced. Mortality not increased. Colony strength and brood not affected.	Pinsdorf, 1989b Study no.: 890620	Already evaluated
Bulldock 25 EC	15 g as/ha + 7.5 g as/ha	Application during foraging in flowering *Phacelia*	Flight intensity sharply decreased for 24-48 hours. Increase in mortality for 1 - 3 days after both applications. No adverse effects on honey bee brood.	Nengel, 1997 Study no.: 97152/01-BFEU	Already evaluated
Bulldock 25 EC	15 g as/ha + 7.5 g as/ha	Application during foraging in flowering *Phacelia*	Foraging activity sharply decreased for 3 days and increased mortality at 15 g as/ha. Foraging activity sharply decreased for 1 day at 7.5 g as/ha and slight increase in mortality. No effects on honey bee brood.	Kleiner, 1997 Study no.: 971048049	Already evaluated

Bulldock 25 EC	2 x 17.5 g as/ha	Evening application on flowering *Phacelia*	Foraging activity reduced for 1 day. Adult mortality not increased. Assessment of colony strength and brood area inconclusive [a]).	Sandrock, 2014c Study no.: 20130101 CP 9.5.1.6/01	**New study**
Bulldock 25 EC	2 x 17.5 g as/ha	Evening application on flowering *Phacelia*	Foraging activity not reduced. Adult mortality slightly increased for 2 days. Colony strength and brood area slightly decreased. No biologically relevant effects on honey bee brood development.	Sandrock, 2014d Study no.: 20120046 CP 9.5.1.6/02	**New study**

[a] Because some colonies showed signs of rearing daughter queens in both, control and test item treatment, followed by queen supersedure in 2 test item replicates, the dataset on colony conditions (brood and number of adult bees) cannot reliably be interpreted.

Report: CP 9.5.1.6/01
Sandrock, C. 2014c
Bulldock 25 EC (as: beta-cyfluthrin) - A Field Study to Evaluate Potential Side Effects on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees, *Apis mellifera* L. (Hymenoptera: Apidae), Following the Application after Bee-Flight on Phacelia tanacetifolia in 2013. Innovative Environmental Services (IES) Ltd, Switzerland report no.: 20130101, edition number: R-33347

Guidelines: PP 1/170 (4). Side-effects on honeybees. Bulletin OEPP/EPPO 40, 313-320. 2010; Partly following elements of OECD Guidance document No. 75 (2007)

GLP: yes

Executive Summary

A field study to evaluate potential side effects on brood development, foraging activity, mortality and behaviour of adult honeybees, *Apis mellifera* L. (Hymenoptera: Apidae), following the application after bee-flight on *Phacelia tanacetifolia* was conducted with Bulldock 25 EC.

The test item was applied twice at an interval of 10 days. For both applications, the treatment rate was 17.5 g beta-cyfluthrin as/ha, corresponding to 700 mL Bulldock 25 EC/ha. Both applications were carried out with a spray volume of 200 L water/ha and were conducted during the *Phacelia* flowering stage BBCH 61 and 65, respectively.
Four colonies serving for biological assessments (foraging, mortality, behaviour, single cell brood development and colony assessments) and 2 colonies serving for collection of samples for residue analysis were placed next to the fields 4 days before the second application. After the second test item application colonies stayed within the fields for another 11 days and were then transferred to a monitoring site, where they were monitored up to day 42.
Exposure of honeybees was confirmed by foraging observations as well as residue analyses on samples of blossoms, pollen and nectar from forager bees, in-hive pollen and nectar, in-hive honey and bee bread.

Exposure to the test item was confirmed by residue analyses of beta-cyfluthrin in blossoms (mean 1.01 mg as/kg), pollen loads collected from forager bees (0.022-0.120 mg as/kg) and from Varroa boards

inserted underneath the hives (probably rejected by the colonies) (0.040-0.390 mg as/kg). Nectar samples extracted from honeybee honey stomachs, as well as all in-hive samples, such as bee bread, nectar and honey, did not contain residues of beta–cyfluthrin above the limit of detection (<0.01 mg/kg).

During the exposure period in the field effects on honeybee behaviour were observed (increased nervousness and aggressiveness). Foraging activity was only affected on the day after the second application but no longer during the 11 days of exposure thereafter.
No effect on forager, drone or pupae mortality was detected during the exposure and post-exposure period.

Development of single brood cells was monitored over 22 days. According to statistical analyses, significantly increased brood termination rates were detected for eggs, young larvae and old larvae (10.4 % 7.2 % and 13.0 %, respectively), as well as significantly decreased brood compensation for young larvae. The brood termination rates observed in the control colonies were very low for all brood stages (3.2 %, 1.7 % and 4.1 % for eggs, young larvae and old larvae, respectively). Although statistically significant differences between control and test item were found in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item are considered of being of marginal biological concern. The brood index and the compensation index were comparable in the control and in the test item treatment. However, because of the very high values combined with low standard deviations, the statistical analysis revealed a significantly lower compensation index for young larvae for the test item.
Because some colonies showed signs of rearing daughter queens in both treatment groups, including queen supersedure in two test item replicates, the dataset on colony conditions should be interpreted with caution, and was therefore split into dataset A (all colonies for biological assessments) and B (dataset A excluding the two test item colonies that exhibited queen supersedure).
While brood nests in the control colonies increased by 11 % during the exposure phase, brood nests of the test item colonies decreased by 34 %.

During the post-exposure phase, brood nests decreased independent of the treatment as a response to the progressing season, yet, this trend was slightly more pronounced in the test item colonies (-77 %) compared to the controls (-50 %) at study termination. Considering colony dataset B, the decrease in the test item group was slightly less after the exposure phase (-30 %) and at study termination (-59 %). Regarding colony dataset A, control colony strength increased by +39 % after the exposure phase and remained at this level at study termination (+41 %). In contrast, colony growth was reduced in the test item treatment colonies, with slightly decreasing worker populations after the exposure phase (-3 %) and only slightly increasing numbers of bees towards the end of the post-exposure phase (+7 %). Considering colony dataset B, the mean relative change of the test item colonies was similar after the exposure phase was (+4 %) but with +15 % at study termination largely in the same range compared to the control.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC (beta-cyfluthrin)
Description:	Colourless limpid liquid
Lot/Batch #:	92110454
Purity:	25 g/L (nominal), 25.8 g/L (measured)

2. Control: not treated

Weather conditions

Transitional exposure period (DAST -4 to 0): unstable conditions with rainfall on DAST -4,-3

and -2 (3 rain events for the control field and 2 rain events for the test item field), but also including extended sunny periods.
Exposure period (DAST 1-11): overall favourable and stable conditions for honeybee flight activity, with rain events only on DAST 2 and 3 in both, control and test item fields.
Post-exposure period (DAST 12 - 42): In general favourable conditions for honeybee flight activity with some rainfall on 12 days.

Test species

Honeybee (*Apis mellifera* L.), healthy honeybee colonies (1 year old queens, deriving from the same breeding strain, but not sister queens) with one hive body including 13-14 Swiss format frames and containing between 20800 to 33100 bees (treatment group averages for the control and test item were 24763 and 25663 bees/colony, respectively), 8 to 11 frames with brood of all stages and at least 3 frames with stores (honey, nectar, pollen).

B: STUDY DESIGN AND METHODS

1. Trial site and test design

Two fields grown with *Phacelia tanacetifolia*, separated by 3.5 km were used. The control field (2.25 ha) was located in CH-4114 Hofstetten and the test item field (2.21 ha) in CH-4118 Rodersdorf, Switzerland.
The test item was applied twice at an interval of 10 days. The *Phacelia* flowering stage was BBCH 61 and 65 at the first and second test item application, respectively. Both applications were conducted after daily bee flight activity during the night. The control field was not treated. Four days before the second test item application 6 colonies were placed at the margins of each field (4 colonies for biological assessments and 2 for residue sampling). After the second test item application (DAST 0), colonies stayed within the fields for another 11 days and were then transferred to a monitoring site during early morning on DAST 12 (before bee flight activity), where they were monitored in the absence of mass-flowering crops and intensive agriculture (DAST 12 to DAST 42).
Residue analyses for beta-cyfluthrin were performed. *Phacelia* flower samples were taken directly after the second night application of the test item (DAST 0). Forager bees were collected at the hive entrances on DAST 0, 1, 2, 3, 4, 7 to examine residues in pollen and nectar collected by the bees. In-hive nectar and pollen samples were collected on DAST 0, 3 and 7, and additional honey samples were taken on DAST 14. *Phacelia* pollen loads that were detected on Varroa boards inserted within hives were collected when noticed in larger quantities (DAST-3 to 7).
For both applications, the treatment rate was 17.5 g beta-cyfluthrin as/ha, corresponding to 700 mL Bulldock 25 EC/ha. Both applications were carried out with a spray volume of 200 L water/ha and were conducted during the *Phacelia* flowering stage BBCH 61 and 65, respectively.

2. Sampling methods and sample processing

Mortality of adult worker honeybees, pupae and larvae was monitored from DAST -4 to DAST 11 in the dead bee traps (in-hive mortality) and on the sheets outspread in the fields (field phase including transitional exposure and exposure phase at the field sites) and DAST 13 to DAST 42 in the dead bee traps (post-exposure phase at the monitoring site). The data obtained on DAST 12 was collected during early morning before bee flight and the colony transfer to the monitoring site, and thus considered as belonging to the exposure phase.
Foraging activity was monitored DAT - 4 to DAT 11 (field phase).
Behaviour of the bees in the crop (DAT -4 to DAT 11) was monitored at the hive entrances and in general (DAT - 4 to DAT 42).
Colony condition assessments (food stores, brood areas, colony strength) of the colonies used for biological assessments were performed on DAST 0, 7, 14, 21, 28, 35 and 42.
Detailed brood assessments (brood termination rate, brood index and brood compensation index of at least 290 marked cells containing eggs, young larvae and old larvae, respectively, resulting in at least 870 marked cells per colony replicate) were performed BFD 0 (DAST 0), BFD 4 (DAST 4), BFD 10 (DAST 10), BFD 16 (DAST 16), BFD 22 (DAST 22).

Details on validation of the analytical method, preparation and processing of samples and actual residue analysis are detailed in the study report.

3. Statistical calculations

Treatment comparisons of mean brood termination rates were performed for consecutive BFD assessments using discrete count data and Fisher's exact tests. Mean brood and compensation indices were compared using two sample tests (either t-test or non-parametric Mann-Whitney analogue, depending on normality and variance distributions).
Treatment mean in-hive mortality was compared for each day and for entire experimental phases (transitional exposure, exposure, post-exposure and post-application) using two sample tests (either t-test or non-parametric Mann-Whitney analogue, depending on normality and variance distributions). Pupae and adult bees were analysed separately and the latter was further separated into workers and drones for additional analyses. Mortality data obtained from the sheets spread out within fields was skewed, as most counts resulted in zeros, and thus not statistically assessed.
Mean foraging activity was assessed per day, based on ten independent observations within fields, and for entire transitional exposure and exposure phases using two sample tests (either t-test or non-parametric Mann-Whitney analogue, depending on normality and variance distributions).
Test direction of the alternative hypothesis: one-sided smaller for mortality (daily and overall comparisons) and brood termination (for each BDF individually), one-sided greater for foraging activity (daily and overall comparisons) and brood and compensation index, respectively (for each BDF individually).
For all tests the significance level was $\alpha = 0.05$.
The following endpoints were evaluated during colony conditions assessments: number of adult bees, comb area comprising of brood (total, as well as separately for eggs, larvae and pupae, respectively), stores, including nectar and honey (combined and separately) and pollen, and vacant cells.
Corresponding data are presented descriptively only, i.e. presenting relative changes compared to the initial assessment.
Two data sets are presented separately:
A) colonies used for biological assessments (N=4 per treatment)
B) dataset A, but excluding test item colony replicates 1 and 2 in which queen supersedure took place during the post-exposure monitoring phase.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-6: Effects of Bulldock 25 EC on honeybee mortality, foraging activity and brood development

Assessment	Control				Test item			
	n = 4				n = 4			
	Worker bees/Colony (mean mortality± SD)							
Transitional exposure (DAST -4 to 0)	9.7	±	3.5		8.0	±	4.1	
Exposure (DAST 0 to 12)	7.0	±	3.0		6.2	±	2.3	
Post- exposure - field (DAST 13 to 42)	6.6	±	1.7		5.5	±	2.9	
Post second application (DAST 0 to 42)	6.7	±	0.8		5.7	±	2.1	
	Drones/Colony (mean mortality± SD)							
Transitional exposure (DAST -4 to 0)	1.3	±	1.0		4.4	±	1.4*	
Exposure (DAST 0 to 12)	2.0	±	1.2		2.3	±	1.6	
Post- exposure (DAST 13 to 42)	0.6	±	0.3		0.5	±	0.1	

Post second application (DAST 0 to 42)	1.0	±	0.5			1.0	±	0.5		
	Pupae/Colony (mean mortality± SD)									
Transitional exposure (DAST -4 to 0)	0.0	±	0.0			1.0	±	0.9*		
Exposure (DAST 0 to 12)	0.2	±	0.4			0.3	±	0.7		
Post- exposure - field (DAST 13 to 42)	0.2	±	0.3			0.3	±	0.3		
Post second application (DAST 0 to 42)	0.2	±	0.2			0.3	±	0.3		
	Worker bees/Sheets (mean mortality ± SD)									
Transitional exposure (DAST -4 to 0)	0.0	±	0.0			0.8	±	1.1		
Exposure (DAST 0 to 12)	0.5	±	1.0			0.5	±	0.9		
	Foraging activity of bees/m^2 (mean ± SD)									
Transitional exposure (DAST -4 to 0)	14.0	±	6.2			12.6	±	4.5		
Exposure (DAST 1 to 11)	10.4	±	2.9			8.1	±	2.7*[a]		
	Mean % of worker population [relative change] [1]									
DAST 0	100					100 (100)				
DAST 7	144					99 (107)				
DAST 14	139					97 (104)				
DAST 21	151					97 (115)				
DAST 29	145					108 (118)				
DAST 35	140					106 (118)				
DAST 42	141					107 (115)				
	Mean % Brood Nest [relative change] [1]									
DAST 0	100					100 (100)				
DAST 7	113					68 (71)				
DAST 14	111					66 (70)				
DAST 21	76					51 (53)				
DAST 29	63					39 (46)				
DAST 35	47					28 (39)				
DAST 42	50					23 (41)				
	Mean Values of Brood Development Eggs ± SD (BFD 22)									
Brood Termination Rate (%) (DAST 22)	3.2	±	1.5			10.4	±	7.7*		
Brood Index (DAST 22)	4.8	±	0.1			4.5	±	0.4		
Compensation Index (DAST 22)	4.9	±	0.1			4.6	±	0.3		
	Mean Values of Brood Development Young Larvae ± SD (BFD 22)									
Brood Termination Rate (%) (DAST 22)	1.7	±	0.9			7.2	±	2.9*		
Brood Index (DAST 22)	4.9	±	0.0			4.6	±	0.2*		
Compensation Index (DAST 22)	4.9	±	0.0			4.7	±	0.1*		
	Mean Values of Brood Development Old Larvae ± SD (BFD 22)									
Brood Termination Rate (%) (DAST 22)	4.1	±	5.2			13.0	±	12.0*		
Brood Index (DAST 22)	4.8	±	0.3			4.4	±	0.6		
Compensation Index (DAST 22)	4.9	±	0.2			4.6	±	0.3		

1	Numbers shown for complete dataset A and dataset B, excluding the two test item colonies in which queen supersedure occurred (in parentheses)
*	Statistically significant difference detected compared to the control, see detailed statistics results
a	No significant difference when DAST 1 is excluded
DAST	Days After Second Treatment exposure
SD	Standard Deviation

Mortality

According to statistical analyses, in-hive mortalities of workers, drones and pupae were not significantly increased in the test item treatment compared to the control during all experimental phases, except for drone and pupae mortality during the transitional exposure phase. There were also no obvious effects on forager mortality (as inferred from the sheets spread out in the fields).

Foraging activity

According to statistical analyses, overall foraging activity was significantly decreased in the test item field during the exposure phase. However, this finding was strongly influenced by the data obtained from DAST 1, where statistical analysis detected highly significant differences. Otherwise foraging levels did not differ between the control and test item treatment throughout the exposure phase.

Behaviour

In the field treated with the test item the following observations were made: (i) short-term repellent effects on the crop after the second test item application; (ii) aggressive and nervous behaviour throughout the exposure phase, and (iii) symptoms of poisoning in the field and at the hive entrances on the day after the second test item application, and occasional indication for clustering at the hive entrance during the exposure phase.
Queen cells were noticed in two control replicates on DAST 7 during the exposure phase, which were, however, no longer present on DAST 14 at the monitoring site. Similarly, on DAST 21 queen cells were noticed in one test item colony replicate that have been removed on DAST 29. However, in two other test item colony replicates queen supersedure was observed in two test item replicates during the post-exposure phase (beginning on DAST 29).

Colony strength

Regarding colony dataset A, control colony strength increased by +39 % after the exposure phase and remained at a high level at study termination (+41 %). In contrast, colony growth was decelerated in the test item treatment colonies, with only slightly increasing worker populations after the exposure phase (-3 %) and only slightly increasing numbers of bees at the end of the post-exposure phase (+7 %). Considering colony dataset B the mean relative change of the test item colonies increased slightly after the exposure phase was (+4 %) and was with +15 % at study termination largely in the same range compared to the control.

Brood nest (eggs/larvae/pupae)

While brood nests in the control colonies increased by 11 % during the exposure phase, brood nests of the test item colonies decreased by 34 %. During the post-exposure phase, brood nests decreased independent of the treatment as a response to the progressing season. Yet, this trend was more pronounced in the test item colonies (-77 %) compared to the controls (-50 %) at study termination. Considering colony dataset B the decrease in the test item group was slightly less after the exposure phase (-30 %) and at study termination (-59 %).

Nectar and honey stores

Nectar and honey stores increased substantially during the exposure phase, suggesting that the bees foraged extensively on the crop. The increase in the test item treatment colonies (+100 %) was stronger compared to the controls (+75 %). During the post-exposure phase at the shared monitoring site this pattern was changed, with control colonies taking advantage of a sporadic honey dew flow in

the surrounding forest more efficiently (+193 %) than test item colonies (+151 %). Considering colony dataset B the relative increase in the test item group was slightly less after the exposure phase (+93 %) and at study termination (+146 %), respectively.

Pollen stores

Pollen stores remained similar in the control group throughout the study (+8 % after the exposure phase and -12 % at study termination). Pollen stores strongly increased in the test item colonies during the exposure phase (+31 %) and the post-exposure phase at the monitoring site (+96 %), which is in accordance with the observation of repellent effects on pollen foraging (but not on nectar foraging) in the test field. Nevertheless, pollen stores were considerably lower in the test item group compared to the control. Considering dataset B the pattern was similar for the test item group (+23 % after the exposure phase and +37 % at study termination).

Brood termination rate

Mean brood termination rates in the control were 3.2 % for eggs, 1.7 % for young larvae and 4.1 % for old larvae and 10.4 % for eggs, 7.2 % for young larvae and 13.0 % for old larvae in the test item, respectively. According to statistical analyses, cumulative mean brood termination rates were significantly higher in the test item treatment compared to the control for all selected brood stages, i.e. eggs, young larvae and old larvae. Acceptability criteria for brood termination rates (BTRs) under field conditions are currently not defined. In the context of the information on commonly observed BTRs available from an evaluation of 17 honeybee brood studies performed according to the Oomen et al. test method across Germany and Switzerland over a period of 15 years, the findings for the test item treatment in the current study, appear to fall in the range found for experimental control colonies of similar strength under field conditions. In this regard, although statistical analyses detected significantly increased brood termination rates for all developmental stages in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item may be considered of being of marginal biological concern.

Brood index

According to statistical analyses, brood indices were significantly lower in the test item treatment compared to the control on at least two of the four assessments for each selected brood stage, i.e. eggs, young larvae and old larvae.

Brood compensation index

According to statistical analyses, mean compensation indices of the test item treatment were significantly lower compared to the control for eggs (BFD 10) and young larvae (BFD 10, 16 and 22) but not for old larvae.

Residue analysis

The mean residue of beta-cyfluthrin in flowers detected in the twelve samples taken at DAST 0 approximately 1 hour after the second test item application was 1.01 mg as/kg and the maximum residue was 1.29 mg as/kg.
Before the second test item application on DAST 0 (transitional exposure, 10 days after the first test item application) the residues of beta-cyfluthrin in pollen loads collected from forager bees were determined to range between 0.028 and 0.034 mg as/kg. After the second test item application the maximum residue in pollen loads collected from forager bees was 0.120 mg as/kg on DAST 1. Later on residues ranged between 0.022 and 0.104 mg as/kg between DAST 2 to 7.
Regarding pollen loads collected from Varroa boards inserted underneath the experimental colonies the two samples collected before the second test item application on DAST -3 and 0 were determined to be 0.060 and 0.044 mg as./kg, respectively. The residue found after the second test item application was determined as 0.390 mg as/kg on DAST 3 and 0.196 mg as/kg on DAST 7.
None of the in-hive samples, such as honey (DAST 14), bee bread and nectar, nor nectar extracted from the honey stomachs of foragers (DAST 0 to 7) contained traceable residues of beta–cyfluthrin (<0.01 mg as/kg).

III. CONCLUSIONS

After two applications of 700 mL Bulldock 25 EC/ha (corresponding to 17.5 g beta-cyfluthrin/ha) onto flowering *Phacelia tanacetifolia* in a 10 day interval the exposure of honeybees to the test item was confirmed by foraging observations as well as residue analyses.
On the day after the second application up to 0.120 mg as/kg were found in pollen loads collected from forager bees but residues detected in pollen loads collected on later sampling occasions declined and ranged between 0.022 and 0.104 mg as/kg. Similarly, residues of beta-cyfluthrin were detected in the pollen loads collected from Varroa boards inserted underneath the hives (probably rejected by the colonies) and ranged between 0.196 and 0.390 mg/kg. However, none of the in-hive samples, such as bee bread, nectar and honey, nor nectar from forager bee stomachs, contained traceable residues of beta–cyfluthrin (<0.01 mg as/kg).
Apparent effects on honeybee behaviour were observed, especially increased nervousness and aggressiveness. Foraging activity was only affected on the day after application but not thereafter. Furthermore, no effect on forager mortality was detected.
According to statistical analyses, significantly increased brood termination rates were detected for all brood stages (eggs, young larvae and old larvae), as well as significantly decreased brood compensation for young larvae. The brood termination rates observed in the control colonies were very low for all brood stages (3.2 %, 1.7 % and 4.1 % for eggs, young larvae and old larvae, respectively), which was probably influenced by the overall large colony strength (all colonies > 20’000 workers). In this regard, although statistical analyses detected significantly increased brood termination rates for all developmental stages in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item (10.4 % 7.2 % and 13.0 % for eggs, young larvae and old larvae, respectively) may be considered of being of marginal biological concern. Because some colonies showed signs of rearing daughter queens in both treatments, including queen supersedure in two test item replicates, the dataset on colony conditions should be interpreted with caution, and was therefore split into dataset A (all colonies for biological assessments) and B (dataset A excluding the two test item colonies that exhibited queen supersedure).
The brood index and the compensation index were comparable in the control and in the test item treatment. However, because of the very high values combined with low standard deviations, the statistical analysis revealed a significantly lower compensation index for young larvae in the test item treatment.
While brood nests in the control colonies increased by 11 % during the exposure phase, brood nests of the test item colonies decreased by 34 %. During the post-exposure phase, brood nests decreased independent of the treatment as a response to the progressing season, yet, this trend was slightly more pronounced in the test item colonies (-77 %) compared to the controls (-50 %) at study termination. Considering colony dataset B, the decrease in the test item group was slightly less after the exposure phase (-30 %) and at study termination (-59 %).
Regarding colony dataset A, control colony strength increased by +39 % after the exposure phase and remained at this level at study termination (+41 %). In contrast, colony growth was decelerated in the test item treatment colonies, with slightly decreasing worker populations after the exposure phase (-3 %) and slightly increasing numbers of bees towards the end of the post-exposure phase (+7 %). Considering colony dataset B, the mean relative change of the test item colonies was similar after the exposure phase was (+4 %) but with +15 % at study termination largely in the same range compared to the control.

RMS’s comments:

This study is considered valid and acceptable for the risk assessment.

Report: CP 9.5.1.6/02
Sandrock, C. 2014d
Bulldock 25 EC (as: beta-cyfluthrin) - A Field Study to Evaluate Potential Side Effects on Brood Development, Foraging Activity, Mortality and Behaviour of Adult Honeybees, Apis mellifera L. (Hymenoptera: Apidae), Following the

Application after Bee-Flight on Phacelia tanacetifolia. Innovative Environmental Services (IES) Ltd, Switzerland, report no: 20120046, edition number: R-28685

Guidelines: OEPP/EPPO Guideline No. 170 (4); Partly following elements of OECD Guidance document No. 75 (2007)

GLP: yes

Executive Summary

A field study to evaluate potential side effects on brood development, foraging activity, mortality and behaviour of adult honeybees, *Apis mellifera* L. (Hymenoptera: Apidae), following the application after bee-flight on *Phacelia tanacetifolia* was conducted with Bulldock 25 EC.

The test item was applied twice at an interval of 10 days. For both applications, the treatment rate was 17.5 g beta-cyfluthrin as/ha, corresponding to 700 mL Bulldock 25 EC/ha. Both applications were carried out with a spray volume of 300 L water/ha and were conducted during the *Phacelia* flowering stage BBCH 61 and 65, respectively.

Four colonies serving for biological assessments (foraging, mortality, behaviour, single cell brood development and colony assessments) and 1 colony serving for collection of samples for residue analysis were placed next to the fields 4 days before the second application. After the second test item application colonies stayed within the fields for another 9 days and were then transferred to a monitoring site, where they were monitored up to day 28. Exposure of honeybees was confirmed by foraging observations as well as residue analyses on samples of blossoms, pollen and nectar from forager bees, in-hive pollen and nectar, in-hive honey and bee bread.

Exposure to the test item was confirmed by residue analyses of beta-cyfluthrin in blossoms (mean 0.645 mg as/kg), pollen loads collected from forager bees (0.011-0.101 mg as/kg) and from Varroa boards inserted underneath the hives (probably rejected by the colonies) (0.085-0.090 mg as/kg). Nectar samples extracted from honeybee honey stomachs, as well as all in-hive samples, such as bee bread, nectar and honey, did not contain residues of beta–cyfluthrin above the limit of detection (<0.01 mg/kg).

Foraging activity was slightly decreased on the day after the second test item application, but overall there was no difference in foraging activity across the entire exposure phase.
According to statistical analyses pupae mortality during the exposure phase was slightly but significantly increased in the test item when compared to control.
No statistically significant difference in pupae mortality between control and test item was found in the post exposure phase. Adult in-hive mortality was slightly but statistically significantly increased. Forager mortality was higher on the day after the second test item application, but across the entire exposure phase there was no statistically significant difference between the two treatments.
Development of single brood cells was monitored over 20 days. The mean cumulative brood termination rate for the observed eggs was moderately increased in the test item group (16.6 %), but overall statistically significantly higher compared to the control (9.9 %). Although statistical analyses detected significantly increased brood termination rates for eggs in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item is considered of being of marginal biological concern. Regarding the brood and the compensation indices, no statistically significant differences were detected.
Regarding colony development, there were minor decreases in the test item on colony strength (-10 %) and brood nest size (-18 %) at the end of the study, while the control displayed relative increases (+11 % and + 15 %, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC (beta-cyfluthrin)
Description:	Yellow-brown, liquid
Lot/Batch #:	0111311
Purity:	25 g/L (nominal), 26.9 g/L (measured)

2. Control: not treated

Weather conditions

Transitional exposure period (DAST -4 to 0): partly unsettled with rainfall on 3 of 5 days and an overall mean temperature of 17.6° C.
Exposure period (DAST 1-9): partly unsettled with rainfall on 6 of 9 days and an overall mean temperature of 16° C.
Post-exposure period (DAST 10 - 28): fairly good, with rainfall on 4 of 19 days and an overall mean temperature of 20.4° C.

Test species

Honeybee (*Apis mellifera* L.), healthy honeybee colonies (sister queens) with one hive body including 12 Swiss format frames and containing between 11200 to 19350 bees (treatment group averages for the control and test item were 17750 and 16038 bees/colony, respectively), 6 to 9 frames with brood of all stages and at least 3 frames with stores (honey, nectar, pollen).

B: STUDY DESIGN AND METHODS

1. Trial site and test design
Two fields grown with *Phacelia tanacetifolia*, separated by 1.6 km, were used. The control field (1.43 ha) was located in 4447 Känerkinden and the test item field (1.51 ha) in 4443 Wittinsburg, Switzerland.
The test item was applied twice with an interval of 10 days. The *Phacelia* flowering stage at the first test item application was BBCH 61-63 and 65 at the second application, respectively. Both applications were conducted after daily bee flight activity during the night. The control field was not treated. Four days before the second test item application 5 colonies were placed at the margins of each test fields (4 colonies for biological assessment and 1 for residue sampling). After the second test item application (DAST 0), colonies remained within fields for 9 days and were then transferred to a monitoring site during the night of DAST 9 (after bee flight activity), where they were monitored in the absence of mass-flowering crops and intensive agriculture (DAST 10 to DAST 28).
For residue analyses, the following matrix was sampled: flower buds (DAST 0 after second test item application), pollen and nectar from forager bees (DAST -1, 1, 2, 3, 4 and 6), in-hive pollen and nectar (DAST -1 for the test item and 0 before second test item application for the control, respectively and on DAST 4 and 6), in-hive honey (DAST 10 or 11).
For both applications, the treatment rate was 17.5 g beta-cyfluthrin as/ha, corresponding to 700 mL Bulldock 25 EC/ha. Both applications were carried out with a spray volume of 300 L water/ha and were conducted during the *Phacelia* flowering stage BBCH 61 and 65, respectively.

2. Sampling methods and sample processing
Mortality of adult worker honeybees, pupae and larvae was monitored from DAST -4 to DAST 9 in dead-bee traps and on the linen sheets (field phase) and DAST 10 to DAST 28 only dead-bee traps (monitoring site phase).
Foraging activity was monitored DAST -4 to DAST 9 (field phase).

Behaviour of the bees in the crop (DAT -4 to DAT 9) was monitored at the hive entrances and in general (DAT - 4 to DAT 28).
Colony condition assessments (food stores, brood areas, colony strength) of the colonies used for biological assessments were performed on DAST -2, DAST 6, DAST 12/13, DAST 19 and DAST 27.
Detailed brood assessments (brood termination rate, brood index and brood compensation index of 398 to 440 selected eggs were performed BFD 0 (DAST -1), BFD 6 (DAST 5), BFD 10 (DAST 9), BFD 17 (DAST 16), BFD 21 (DAST 20).
Details on validation of the analytical method, preparation and processing of samples and actual residue analysis are detailed in the study report.

3. Statistical calculations
Welch's t-test and in the case of non-normality of parent distribution non-parametric Mann-Whitney test. In the case of brood termination where observations were recorded as discrete counts, Fisher's exact test was used. Test direction of the alternative hypothesis: one-sided smaller for mortality (daily and overall comparisons) and brood termination (daily), one-sided greater for foraging activity (overall comparisons) and brood and compensation index, respectively (daily).
Significance level: For all data: $\alpha = 0.05$

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-7: Effects of Bulldock 25 EC on honeybee mortality, foraging activity and brood development

Assessment	Control				Test item			
	n = 4(5)[a]				n = 4(5)[a]			
	Worker bees/Colony (mean mortality± SD)							
Transitional exposure (DAST -4 to 0)	19.3	±	6.3		29.1	±	16.9	
Exposure (DAST 1 to 9)	11.2	±	8.8		19.8	±	17.9	*
Post-exposure (DAST 10 to 28)	56.5	±	16.7		78.2	±	41.1	*
Post-2nd-application (DAST 1 to 28)	41.9	±	11.7		59.4	±	28.6	*
	Pupae/Colony (mean mortality± SD)							
Transitional exposure (DAST -4 to 0)	0.8	±	1.7		1.0	±	0.8	
Exposure (DAST 1 to 9)	0.7	±	1.8		1.1	±	1.7	*
Post-exposure (DAST 10 to 28)	0.3	±	0.4		0.3	±	0.3	
Post-2nd-application (DAST 1 to 28)	0.4	±	0.5		0.6	±	0.5	
	Worker bees/Sheets (mean mortality ± SD)							
Transitional exposure (DAST -4 to 0)	2.4	±	2.1		0.2	±	0.4	
Exposure (DAST 1 to 9)	1.1	±	1.7		3.0	±	5.1	
	Foraging activity of bees/m^2 (mean ± SD)							
Transitional exposure (DAST -4 to 0)	11.2	±	6.9		11.4	±	6.1	
Exposure (DAST 1 to 9)	6.8	±	6.7		7.4	±	6.0	
	Mean values of Brood Development							
Brood termination rate (%) BFD 21 (DAST 20)	9.9	±	5.7		16.6	±	12.0	Δ
Brood index BFD 21 (DAST 20)	4.5	±	0.3		4.2	±	0.6	
Compensation index BFD 21 (DAST 20)	4.6	±	0.2		4.3	±	0.6	

a For brood termination and brood and compensation index colony replicates used for residue sampling were included
* Statistically significantly greater when compared to the control (Mann-Whitney, α=0.05, alternative one sided smaller)
Δ Statistically significantly greater when compared to the control (Fisher's exact test, α=0.05, alternative one-sided smaller)
DAST Days After Second Treatment

Table B.9.5-8: Effects of Bulldock EC 25 on colony strength, brood nest, stores and vacant cells

Assessment	Control					Test Item			
	Mean	±	SD	Change in %		Mean	±	SD	Change in %
	Mean colony strength[1)]								
Transitional Exposure DAST -2	17750	±	2313	100		16038	±	3489	100
Exposure DAST 6	19763	±	2781	111		16563	±	3775	103
Post-Exposure DAST 12	22775	±	4644	128		16875	±	4327	105
Post-Exposure DAST 19	20813	±	4843	117		16713	±	4576	104
Post-Exposure DAST 27	19638	±	6909	111		14513	±	4341	90
	Mean comb area[2)] (cm²) of brood nest (eggs, larvae, pupae) / colony								
Transitional Exposure DAST -2	5917	±	1621	100		6700	±	1526	100
Exposure DAST 6	6954	±	1948	118		6366	±	818	95
Post-Exposure DAST 12	7172	±	1891	121		6240	±	718	93
Post-Exposure DAST 19	6689	±	1366	113		5802	±	932	87
Post-Exposure DAST 27	6804	±	1602	115		5468	±	1189	82
	Mean comb area[2)] (cm²) of stores (nectar, honey, pollen) / colony								
Transitional Exposure DAST -2	8968	±	2577	100		7840	±	1199	100
Exposure DAST 6	7817	±	3078	87		7046	±	883	90
Post-Exposure DAST 12	6746	±	2961	75		6240	±	940	80
Post-Exposure DAST 19	7322	±	2675	82		7368	±	1462	94
Post-Exposure DAST 27	7046	±	2034	79		7472	±	1415	95
	Mean comb area[2)] (cm²) of vacant cells / colony								
Transitional Exposure DAST -2	7218	±	1356	100		7564	±	1522	100
Exposure DAST 6	7333	±	1386	102		8692	±	1306	115
Post-Exposure DAST 12	8185	±	1824	113		9624	±	697	127
Post-Exposure DAST 19	8093	±	1600	112		8934	±	2065	118
Post-Exposure DAST 27	8254	±	439	114		9164	±	2077	121

[1)] Number of bees per colony estimated according to Liebefelder method.
[2)] Comb area per frame side = 921 cm² corresponding to 22104 cm² of a colony with 12 frames.

Mortality (adult worker bees)

Transitional exposure (field site) DAST -4 to 0

Mean in-hive adult bee mortality (dead bee traps) per colony and day during the days before the second test item application was 19.3 dead bees in the control and 29.1 dead bees in the test item group. No statistically significant difference was detected.
Mean forager mortality, as inferred from sheets spread out within fields, was 2.4 bees/sheet in the control field and 0.2 bees/sheet in the test item. Across the entire transitional exposure phase no statistically significant difference was detected.

Exposure (field site) DAST 1 to 9

According to statistical analyses mean adult bee mortality per day (dead-bee traps) was significantly increased in the test item (19.8 bees/colony) compared to the control (11.2 bees/colony) during the exposure phase after the second test item application. Statistically significantly increased mortality in the test item group was noticed on DAST 1, 2, 6.
The overall daily mean forager mortality was 1.1 bees/sheet in the control field and 3.0 bees/sheet in the test item field. Across the entire exposure phase no statistically significant difference was detected. On DAST 1, the day after the second test item application, forager mortality in the test item field was increased (16 bees/sheet) compared to the control field (0 bees/sheet).

Post exposure (DAST 10 to 28) (monitoring site) and post second application (DAST 1 to 28)

According to statistical analyses mean adult bee mortality per day (dead bee traps) during the post exposure phase was significantly increased in the test item compared to the control on DAST 12, 13. Overall statistics detected significantly increased mean adult bee mortality in the test item compared to the control across the entire post exposure phase (78.2 and 56.5 bees/colony, respectively) and across the entire post second application phase (59.4 and 41.9 bees per colony, respectively).

Mortality (pupae)

Transitional exposure DAST -4 to 0

Mean pupae mortality (dead bee traps) per day during the days before the second test item was similar in both treatments (0.8 to 1.0 pupae per colony per day for the control and test item treatment, respectively).

Exposure (field site) DAST 1 to 9

During the exposure phase after the second test item application the mean number of dead pupae was low overall. Regarding individual days, no statistically significant differences were detected. Statistical analyses across the entire exposure phase detected significantly increased mean pupae mortality in the test item (1.1 pupae/colony) compared to the control (0.7 pupae/colony).

Post exposure (DAST 10 to 28) (monitoring site) and post second application (DAST 1 to 28)

Mean pupae mortality remained at a low level in both treatments throughout the post exposure phase (0.3 and 0.3 pupae/colony for the control and test item, respectively) and throughout the post second application phase (0.4 and 0.6 pupae/colony for the control and test item, respectively). No statistically significant differences were detected.

Mortality (larvae)

No larvae mortality was observed during the course of the study.

Foraging activity

Transitional exposure DAST -4 to 0

Mean foraging activity was similar in both, the control field (11.2 bees/m^2) and the test item field (11.4 bees/m^2), indicating that the crop was attractive to the bees.

Exposure (field site) DAST 1 to 9

After the second test item application mean foraging activity decreased overall but was similar in both fields (6.8 and 7.4 bees per m^2 for the control and test item treatment, respectively). Statistical analyses for the individual foraging assessments on the first day after the second night application of the test item detected significantly decreased foraging activity in the test item treatment for 3 out of 4 foraging assessments (during morning and afternoon, but not around noon). Further, on DAST 6 foraging in the test item field was statistically significantly lower compared to the control. However, no statistically significant difference was detected across the entire exposure phase.

Behaviour

The day after the second test item application (DAST 1) some bees with symptoms of poisoning were noticed in the dead bee traps and on the sheets spread out within fields. No direct repellent effect on foraging activity was observed during the exposure phase (DAST 1 to 9). On DAST 1 foraging activity in the test item field was reduced compared to the control field.
Between DAST 1 and 3 an increased number of *Phacelia* pollen loads were found on the *Varroa* boards inserted underneath the test item colonies (probably rejected within the hives). Furthermore, the mean comb area (cm^2) covered with *Phacelia* pollen was lower in the test item colonies for the colony condition assessments on DAST -2 and 6 (789 and 842 cm^2) when compared to the colonies in the control field (2122 and 2340 cm^2). The rejection of collected *Phacelia* pollen within the colonies and the reduced foraging of *Phacelia* pollen may be interpreted as repellent effect on foraging and storage behaviour of honeybees caused by pollen contaminated with Bulldock 25 EC.

Colony strength

The mean colony strength before the second test item application (DAST -2) was 17750 and 16038 bees/colony in the control and test item treatment, respectively, and thus similar in both treatments. Shortly after the exposure phase (DAST 12) the relative increase in colony strength compared to DAST -2 was +28 % and +5 % in the control and test item treatment, respectively. During the course of the study (DAST -2 to DAST 27), the mean colony strength in the control displayed a relative increase of 11 % while the test item displayed a relative decrease of 10 %, corresponding to 19638 and 14513 bees/colony, respectively, at DAST 27.

Brood nest (eggs/larvae/pupae)

At the 1st assessment (DAST -2) a healthy queen was present in each colony and the brood nest (including eggs, larvae and pupae) was similar in both treatments with mean comb areas comprising of brood of 5917 and 6700 cm^2/colony for the control and test item, respectively.
Corresponding mean comb areas for eggs, larvae and pupae were 1128 and 1382 cm^2/colony, 1474 and 2015 cm^2/colony, and 3316 and 3304 cm^2/colony for the control and test item treatment, respectively.
Shortly after the exposure phase (DAST 12) the relative increase in brood nest size compared to DAST -2 was +21 % and -7 % in the control and test item treatment, respectively.
Corresponding relative changes of mean comb areas for eggs, larvae and pupae were +30 and -2 %, +24 and -25 %, and +17 and +2 % for the control and test item treatment, respectively.
During the course of the study (DAST -2 to DAST 27), the mean brood nest size of the control displayed a relative increase of 15 %, while a relative decrease of 18 % was documented for the test item at DAST 27.
Corresponding relative changes of mean comb areas for eggs, larvae and pupae were +32 and -32 %, +27 and -34 %, and +4 and -3 % for the control and test item treatment, respectively.

Stores (pollen/nectar/honey)

The mean comb area comprising of pollen per colony before the second test item treatment application (DAST -2) was 2383 and 886 cm^2/colony in the control and test item treatment, respectively.
After the exposure phase at DAST 12, mean comb area comprising of pollen in the control and test item treatment displayed a relative change -15 and +49 %, respectively, compared to DAST -2.

At the end of the monitoring phase at DAST 27, mean comb area comprising of pollen in the control and test item treatment displayed a relative change -42 and +51 %, respectively, compared to DAST -2, corresponding to 1382 and 1335 cm^2/colony, respectively.
Accordingly, the initial difference in pollen stores between the control and the test item treatment that may have been influenced by repellent effects of the test item itself during the transitional exposure phase were compensated after 2.5 weeks at the monitoring site.
The mean comb area comprising of nectar and honey (combined) per colony before the second test item treatment application (DAST -2) was 6585 and 6954 cm^2/colony in the control and test item treatment, respectively.
After the exposure phase at DAST 12, mean comb area comprising of nectar and honey (combined) in the control and test item treatment displayed a relative change -28 and -29 %, respectively, compared to DAST -2.
At the end of the monitoring phase at DAST 27, mean comb area comprising of nectar and honey (combined) in the control and test item treatment displayed a relative change -14 and -12 %, respectively, compared to DAST -2, corresponding to 5664 and 6136 cm^2/colony, respectively.

Brood termination rate

The mean brood termination rate of selected eggs at the last brood assessment (DAST 20/BFD 21) was 9.9 % for the control (ranging from 3.8 to 19.0 %) and 16.6 % for the test item (ranging from 4.8 to 30.5 %). According to statistical analyses, compared to the control, mean brood termination rates of the test item were significantly higher at each BFD assessment. Acceptability criteria for brood termination rate (BTR) under field conditions are currently not defined. In the context of the information on commonly observed BTRs available from an evaluation of 17 bee brood studies performed according to the Oomen *et al.* test method across Germany and Switzerland over a period of 15 years, experimental control colonies of variable strength were found to exhibit BTRs of 23.8 % on average. Although colonies containing more than 10’000 workers tend to exhibit BTRs lower than 20 % more frequently, the findings for the test item treatment in the current study appear to fall in the range observed for experimental control colonies of similar strength. In this regard, although statistical analyses detected significantly increased brood termination rates in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item may be considered of being of marginal biological concern.

Brood index

Mean brood indices of the control and test item were very similar across all BFDs. On the last assessment (DAST 20/BFD 21) the mean brood index was 4.5 and 4.2 for the control and test item, respectively. No statistically significant difference was detected.

Brood compensation index

Mean compensation indices of the control and test item were very similar across all BFDs. On the last assessment (DAST 20/BFD 21) the mean compensation index was 4.6 and 4.3 for the control and test item, respectively. No statistically significant difference was detected.

Residue analyses

The mean residue of beta-cyfluthrin in flowers detected in the twelve samples taken at DAST 0 approximately 1 hour after the second test item application was 0.645 mg as/kg and ranged between 0.358 and 1.152 mg as/kg.
In the sample of pollen loads collected from forager bees taken on the day before the second test item application (DAST -1, transitional exposure, 10 days after the first test item application) no residues of beta-cyfluthrin were detected. After the second test item application residues detected in pollen loads collected from forager bees were 0.075 mg as/kg (DAST 1), 0.101 mg as/kg (DAST 2), 0.045 mg as/kg (DAST 3), 0.011 mg as/kg (DAST 4) and 0.014 mg as/kg (DAST 6).
Regarding pollen loads collected from *Varroa* boards inserted underneath the experimental colonies the residues of beta-cyfluthrin were determined to be 0.090 mg as/kg (DAST 1), 0.085 mg as/kg (DAST 2) and 0.087 mg as/kg (DAST 3).

Nectar samples extracted from honeybee honey stomachs did not contain traceable residues of beta-cyfluthrin except the sample taken at DAST -1, which was determined to be 0.009 mg as/kg after correction for matrix effects.
None of the in-hive bee bread, nectar and honey samples contained traceable residues of beta–cyfluthrin (<0.01 mg/kg).

III. CONCLUSIONS

To assess the potential effects of Bulldock 25 EC on honeybee (*Apis mellifera* L.) mortality foraging activity, behaviour and brood development, the test item was applied at a rate of 700 mL Bulldock 25 EC (corresponding to 17.5 g beta-cyfluthrin/ha) on flowering *Phacelia tanacetifolia* twice with an interval of 10 days after bee flight under field conditions in summer 2012.

Exposure to the test item was confirmed by residue analyses of beta-cyfluthrin in pollen loads collected from forager bees (0.011-0.101 mg as/kg) and pollen loads collected from Varroa boards inserted underneath the hives (probably rejected by the colonies) (0.085-0.090 mg as/kg). Nectar samples extracted from honeybee honey stomachs, as well as all in-hive samples, such as bee bread, nectar and honey, did not contain residues of beta–cyfluthrin above the limit of detection (<0.01 mg/kg).
The brood termination rate was moderately increased in the test item group, but overall statistically significantly higher compared to the control across all brood assessments. Cumulative mean brood termination rates on the last assessment days were 9.9 % and 16.6 % in the control and test item, respectively. In this regard, although statistical analyses detected significantly increased brood termination rates for eggs in the test item treatment compared to the control, the overall level of brood termination rates observed in the test item (16.6 % for eggs) can be considered of being of marginal biological concern. Regarding the brood and the compensation indices, no statistically significant differences were detected.
According to statistical analyses pupae mortality during the exposure phase was slightly but significantly increased in the test item when compared to control. No statistically significant difference in pupae mortality between control and test item was found in the post exposure phase. Adult in-hive mortality was slightly but statistically significantly increased during the exposure phase, the post-exposure phase and the entire period after the second test item application. Forager mortality was higher on the day after the second test item application, but across the entire exposure phase there was no statistically significant difference between the two treatments.
Foraging activity was slightly decreased on the day after the second test item application, but overall there was no difference in foraging activity across the entire exposure phase.
Regarding colony development, there were minor effects of the test item on colony strength (-10 %) and brood nest size (-18 %) at the end of the study, while the control displayed relative increases (+11 % and +15 %, respectively).

RMS's comments:
There are limitations for interpretability as test fields were separated only by 1.6 km. Therefore, this study is considered not sufficiently valid and serves as additional information only.

B.9.5.1.7 Summary of effects on honeybees

Due to the results of laboratory tests Bulldock EC 25 is considered toxic to bees for oral as well as contact toxicity. Hazard quotients are clearly above the trigger of 50.
In semi-field studies at all application rates, bee flight intensity was transiently reduced when Bulldock 25 EC was applied after bee flight. In addition, behavioural effects and higher mortality rates were observed during the first 2 days following the evening application.
In field studies the foraging activity was still transiently reduced with no effects on colony strength or bee brood development when Bulldock 25 EC was applied after bee flight. When Bulldock 25 EC was applied during bee flight the foraging activity was decreased for 1 day with a slight increase in

mortality and still no effects on colony strength or bee brood development. However, recent field studies with Bulldock 25 EC applied after bee flight show effects on brood development and brood termination rate in the test item treatment which may be related to the treatment.
Therefore, it can be concluded that Bulldock 25 EC has to be classified as hazardous to bees and must not be used on plants which are in flower or which are visited by bees; this also applies to weeds and honey dew.

B.9.5.1.8 Risk assessment for honeybees

Existing data on honeybees were assessed during the EU evaluation of beta-cyfluthrin (2002). Reference is made to the studies used for Annex I inclusion of beta-cyfluthrin which are considered appropriate for the renewal of approval of beta-cyfluthrin. In order to complete this data set, one acute toxicity test on adult honeybees, one acute toxicity test on honeybee larvae and one chronic adult oral feeding test as well as two new honeybee field studies were conducted with Bulldock 25 EC.

Toxicity

The active substance beta-cyfluthrin is of high toxicity to honey bees after acute oral and contact exposure (LD_{50} of about 0.05 µg/bee and 0.01 µg/bee, respectively), the product Bulldock 25 EC is of comparable high oral and contact toxicity (0.0337 µg as/bee and 0.0164 µg/bee respectively).

Table B.9.5-9: Acute toxicity of Bulldock 25 EC, beta-cyfluthrin, cyfluthrin and to bees

Test substance	Test species	Endpoint	Value	Reference
Bulldock 25 EC	honeybee	48 h acute oral LD_{50}	0.0164 µg as/bee	Schmitzer and Sekine, 2010 * Project 52601035
		96 h acute contact LD_{50}	0.0337 µg as/bee	
Bulldock 25 EC	honeybee	72 h acute oral LD_{50}	< 0.025 µg as/bee	Pinsdorf, 1987 SANCO/6841/VI/97-final**
		72 h acute contact LD_{50}	-	
Bulldock 25 EC	honeybee	72 h acute oral LD_{50}	< 0.25 µg as/bee	Stute, 1987 SANCO/6841/VI/97-final**
		72 h acute contact LD_{50}	-	
Bulldock 25 EC	honeybee	72 h acute oral LD_{50}	< 0.25 µg as/bee	Mautz, 1987 SANCO/6841/VI/97-final**
		72 h acute contact LD_{50}	-	
beta-cyfluthrin technical	honeybee	48 h acute oral LD_{50}	0.05 µg as/bee	Kleiner, 1996 SANCO/6841/VI/97-final
		48 h acute contact LD_{50}	0.012 µg as/bee	
cyfluthrin technical	honeybee	48 h acute oral LD_{50}	0.051 µg as/bee	Davies *et al*., 1985 SANCO/6841/VI/97-final
		48 h acute contact LD_{50}	0.0098 µg as/bee [1]	

[1] reported value of 0.001 µg/bee in the review report is considered a typographical error

***** new study submitted for the re-evaluate of beta-cyfluthrin

** results of laboratory bee toxicity studies according to the BBA guideline VI, 23-1

Exposure

The current use pattern for Bulldock 25 EC comprises field applications on wheat or potatoes (application rates 2 x 7.5 g as/ha, 2 x 12.5 g as/ha) and glasshouse applications on tomatoes (application rates 17.5 g as/ha).
The applications in potato fields are recommended between BBCH 10 and BBCH 49 were as application in wheat is recommended between BBCH 10 and BBCH 75.
In case of application in tomatoes in glasshouses (BBCH 7- harvest), no exposure of honey bees and other pollinators is expected, except for commercial pollinators used in glasshouses.

Acute risk to honeybees

The acute risk to honeybees from use of Bulldock 25 EC was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients according to the EPPO risk assessment scheme as follows:

Q_H = Application rate [g as/ha] / LD_{50} oral / contact [µg as/bee]

Hazard quotients were calculated for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) to Bulldock 25 EC with the individually applied dose of 12.5 g as/ha in wheat or potatoes and 7.5 g as/ha in tomatoes. The results are shown in Table B.9.5-10.

Table B.9.5-10: Risk to bees from exposure to Bulldock 25 EC

Substance	Crop	Application rate [g as/ha]	Exposure route	LD_{50} [µg as/bee]	Hazard quotient
Bulldock 25 EC	Tomatoes	17.5	Contact	0.0337	519
			Oral	0.0164	1067
	Wheat, potato	12.5	Contact	0.0337	371
			Oral	0.0164	762

All hazard quotients are above the trigger value of 50, indicating that the active substances pose a high risk to bees. Therefore, a higher tier risk assessment is presented below.

Toxicity to bee larvae and chronic toxicity to adult honeybees

According to current regulations an acute feeding study on honeybee larvae and a chronic feeding study on adult honey bees were conducted with the representative formulation Bulldock 25 EC.
In both studies the determined LD_{50} value via oral exposure (larvae LD_{50} = 0.020 µg as/larvae; adult chronic LD_{50} = 0.019 µg as/bee/day) was in the same range as the acute oral toxicity for Bulldock 25 EC (LD_{50} = 0.0164 µg as/bee).
Thus, there is no indication of a higher sensitivity of adults from chronic compared to acute exposure and no indication of a higher sensitivity of larvae compared to adults.

Higher tier risk assessment for honeybees

A number of semi-field (tent/tunnel) and field studies is available and is considered in the following risk assessment conclusion on the general safety of beta-cyfluthrin to bees resulting from field applications of Bulldock 25 EC.

Semi-field (tunnel/tent) studies under confined conditions

In seven semi-field studies, 0.15 % Bulldock 25 EC (equivalent to 15 g as/ha, Pinsdorf 1989a, Pinsdorf 1989b, Schulz 1989a, Schulz 1989b) and 0.375 % Bulldock 25 EC (equivalent to 37.5 g as/ha, Stute 1987a, Stute 1987b) was applied on flowering *Phacelia* in the evening after bee flight. At all application rates, bee flight intensity was transiently reduced. In addition, behavioural effects and higher mortality rates were observed during the first 2 days following the evening application. Therefore, higher tier studies are required.

Field studies

In four field studies which had already been assessed during the first EU evaluation of beta-cyfluthrin (2002), 0.15 % Bulldock 25 EC (equivalent to 15 g as/ha) was applied on flowering *Phacelia* fields in the evening after bee flight (Stute 1989a, Stute 1989a, Pinsdorf 1989c, Pinsdorf 1989d). Here the foraging activity was still transiently reduced (refer to semi-field). However the mortality, the colony strength and brood were not affected.
Furthermore two field studies have been assessed during the first EU evaluation to address potential effects of Bulldock 25 EC on bee colonies when applied to flowering crops during bee flight (Nengel 1997, Kleiner 1997). In both studies Bulldock 25 EC was applied at rates of 7.5 and 15 g as/ha to flowering *Phacelia* fields. There were sharp decreases in foraging activity and increased mortality for up to 3 days after applications at 15 g as/ha. At 7.5 g as/ha foraging activity was decreased for 1 day with a slight increase in mortality. There were no effects on bee brood in both studies for both application rates.
In two new field studies (Sandrock 2014c, Sandrock 2014d) honeybees were monitored after two applications of 17.5 g beta-cyfluthrin as/ha (700 mL Bulldock 25 EC/ha) after daily bee flight onto flowering *Phacelia tanacetifolia* in a 10-day interval. In the first study of Sandrock (2014c) the foraging activity was affected on the day after the second application and no effect on forager mortality was detected. Apparent effects on honeybee behaviour were observed, especially increased nervousness and aggressiveness. Furthermore, during the exposure phase and the post-exposure phase, brood nests decreased more in the test item colonies compared to the control colonies. However, some colonies showed signs of rearing daughter queens in both treatments, including queen supersedure in two test item replicates. Therefore the dataset on colony conditions should be interpreted with caution. In the second study of Sandrock (2014d) the foraging activity was not affected, but adult mortality slightly increased for 2 days. The colony strength and brood nest area remained in largely the same range over the exposure and post-exposure monitoring period. However, the distance between control and test item field was only 1.6 km in the study 2012, which is considered not sufficient and thus reliability of the test is considered limited. On the basis of the available data, effects on colony conditions cannot finally be excluded.

Overall conclusions of risk to bees

Due to the results of laboratory tests Bulldock EC 25 is considered highly toxic to bees for oral as well as contact toxicity. Hazard quotients are clearly above the trigger of 50 indicating a high potential risk. As observed in semi-field studies on bees, beta-cyfluthrin has adverse effects on bee mortality when applied on flowering crops during daily bee flight. In addition, behavioural effects and slightly increased mortality rates were observed during the first 2 days following the evening application. Therefore, higher tier studies are required.
In the field studies reported for the evaluation in 2002, with application of 15 g as/ha to flowering *Phacelia* fields in the evening after the bee flight activity foraging activity was still transiently reduced and mortality was not increased. However, in field studies conducted during bee flight the flight intensity was reduced and mortality was slightly increased for one day (7.5 g as/ha) or 3 days (15 g as/ha) after application.

In the new field studies (Sandrock 2014c, Sandrock 2014d) with application after daily bee flight and repeated application at 17.5 g as/ha the foraging activity was reduced for 1 day and adult mortality slightly increased for 2 days. Furthermore, effects on brood development and brood termination rate were observed in the test item treatment.
Based on the total set of data, it can be concluded that Bulldock 25 EC has to be classified as hazardous to bees. Therefore it must not be used on plants which are in flower or which are visited by bees; this also applies to weeds and honey dew.

B.9.5.2Effects on non-target arthropods other than bees

Existing data on non-target arthropods were assessed during the EU evaluation of beta-cyfluthrin (2002). Reference is made to the studies used for Annex I inclusion of beta-cyfluthrin.
However, re-evaluating the studies, many of them turn out to be not valide and/or not plausible.
For detailed reasons please refer to the documentation of the respective study in section B.9.5.2.
Two new tier 1 tests on glass plates with Typhlodromus pyri and Aphidius rhopalosiphi are available (see section B.9.5.2.1) as well as three aged residue studies with *Coccinella septempunctata* (see section B.9.5.2.2) , two in-field studies with the formulation Bulldock 25 EC, two in-field studies with a cyfluthrin-formulation and one off-field study with Bulldock 25 EC (see B.9.5.2.4)

The following summary is limited to the laboratory studies (glass-plates, extended studies, aged residues) as well as semi-field studies.

Table B.9.5-11: Summary of all submitted laboratory, extended laboratory, aged residue and semi-field tests with non-target arthorpods other than bees

Species	Treatment rates (g as/ha) Results	Reference	reliability
laboratory test (tier 1 – glass plates)			
Typhlodromus pyri	LR50 = 0.0025 g as/ha 20 %, 28 %, 41 %, 82 % and 100 % mortality at 0.3, 0.9, 2.7, 8.1 and 24.3 mg as/ha	KIIIA1 10.5.1/02 FC010TPL Roig, 2014a M-479587-02-1 R-33356	valid for assessing mortality; effects on reproduction were not investigated
Aphidius rhopalosiphi	LR50 = 0.163 g as/ha 3 %, 5 %, 25 %, 29 % and 90 % mortality at 20, 40, 80, 160 and 320 mg as/ha	KIIIA1 10.5.1/01 FC011ARL Roig, 2014b M-479582-01-1 R-33355	valid for assessing mortality; effects on reproduction were not investigated
Poecilus cupreus	7.7 g as/ha Mortality: 0 % Slight effects on food consumption up to 2 days after application	KIIIA1 10.5.1/01 HBF/CA 27 Heimbach, 1990 M-052707-01-1 R-19124	
extended laboratory test (tier 2)			
Typhlodromus pyri	LR50 = 0.24 g as/ha detached apple leaves 96 % and 98 % mortality at 1.0 and 0.6 g as/ha, respectively; 46 %, 21 % and 26 % mortality at 0.3, 0.2 and 0.1 g as/ha, respectively; Reduced reproduction rate of 25 %, 45 % and 78 % at 0.1, 0.2 and 0.3 g as/ha, respectively	KIIIA1 10.5.2/03 B043TPE Aldershof, 1999 M-022573-01-1 R-19120	not valid

<table>
<tr><td>Aphidius rhopalosiphi</td><td colspan="4">70 g as/ha
100 % mortality of exposed pupae</td><td>KIIIA1 10.5.2./01
97 10 48 004
Kleiner, 1997
M-052305-01-1
R-19126</td><td>not appropriate</td></tr>
<tr><td>Aphidius rhopalosiphi</td><td colspan="4">LR50 = 17.0 g as/ha
52 % and 30 % mortality at 17.0 and 5.4 g as/ha, respectively;
18 %, 16 % and 3.5 % mortality at 1.7, 0.7, 0.2 g as/ha, respectively;
No reproductive effects up to the highest tested rate of 1.7 g as/ha</td><td>KIIIA1 10.5.2./05
B042ARE
Aldershof, 1999
M-015321-01-1
R-19122</td><td>not valid</td></tr>
<tr><td>Coccinella septempunctata</td><td colspan="4">LR50 = 0.0261 g as/ha
70 % and 39 % mortality at 0.05 and 0.025 g as/ha, respectively;
28 %, 39 % and 21 % mortality at 0.01, 0.005 and 0.0025 g as/ha, respectively;
No reproductive effects up to the tested rate of 0.025 g as/ha.</td><td>KIIIA1 10.5.2./04
99 10 48 120
Kleiner, 2001
M-032166-01-1
R-19125</td><td>not valid</td></tr>
<tr><td>Chrysoperla carnea</td><td colspan="4">9.68 g as/ha
100 % mortality</td><td>KIIIA1 10.5.1./05
92022/01-CC
Kuehner, 1993
M-052746-01-2
R-19127</td><td>not valid</td></tr>
<tr><td>Aleochara bilineata</td><td colspan="4">14.2 g as/ha
25.4 % effect on mortality
20 % reduction in parasitation rate</td><td>KIIIA1 10.5.1./02
SXR/AL 11
Schmuck, 1992
M-052616-01-1
R-19128</td><td>not valid</td></tr>
<tr><td>Poecilus cupreus, larvae formulation mixed into soil</td><td>Conc. [mg as/kg soil]</td><td>Morta-lity [%]</td><td>Effect on weight [%]</td><td>develop mental time [d]</td><td>KIIIA1 10.5.2./02
Neumann (2001)
M-080415-01-1</td><td>valid</td></tr>
<tr><td></td><td>Control</td><td>10</td><td>–</td><td>41.0</td><td></td><td></td></tr>
<tr><td></td><td>0.040</td><td>0</td><td>8.2</td><td>49.1</td><td></td><td></td></tr>
<tr><td></td><td>0.4</td><td>100</td><td>–</td><td>–</td><td></td><td></td></tr>
<tr><td></td><td>4.0</td><td>100</td><td>–</td><td>–</td><td></td><td></td></tr>
<tr><td colspan="7">aged residue studies</td></tr>
<tr><td>Coccinella septempunctata</td><td colspan="4">2 applications at drift rates on potted bean plants – leaves removed for bioassays:
Mortality
0.3 g as/ha: 0 day – 91 %
3 days – 64 %
7 days – 87 %
14 days – 11 %
1.2 g as/ha: 0 day – 100 %
3 days – 100 %
7 days – 100 %
14 days – 19 %
Reproductive effects (compared to control)
14 days 0.3 g as/ha:
fertile eggs/female/day +218 %
Larval hatching rate -7 %
14 days 1.2 g as/ha:
fertile eggs/female/day -79 %
Larval hatching rate -23 %</td><td>KIIIA1 10.5.2./07
18121013 Moll, 2004a R-19424</td><td>valid</td></tr>
</table>

Coccinella septempunctata	2 applications at drift rates on potted bean plants – leaves removed for bioassays: Mortality 12.5 g as/ha: 0 day – 100 % 14 days – 69 % 28 days – 30 % Reproductive effects (compared to control) 28 days 12.5 g as/ha: fertile eggs/female/day -11 % Larval hatching rate +30 %	KIIIA1 10.5.2./08 18122013 Moll, 2004b R-19425	valid
Coccinella septempunctata	2 applications at drift rates on potted bean plants – leaves removed for bioassays: Mortality 20 g as/ha: 0 day – 100 % 28 days – 46.2 % 42 days – 21.1 % Reproductive effects (compared to control) 28 days 20 g as/ha: fertile eggs/female/day +86 % Larval hatching rate +7 % 42 days 20 g as/ha: fertile eggs/female/day -18 % Larval hatching rate +13 %	KIIIA1 10.5.2./09 25141013 Moll, 2005a R-19594	valid
Semifield studies			
Poecilus cupreus	8.3 g as/ha in oilseed rape No significant effects on mortality	KIIIA1 10.5.3/03 SXR/HF 91 Schmuck, 1993 M-052542-01-1 R-19129	not valid
Poecilus cupreus	12.5 g as/ha in orchards No significant effects on mortality Sublethal effects (feeding rate) 100 % in adults 3 days after treatment	KIIIA1 10.5.2/03 SXR/HF 88 Schmuck, 1993 M-052590-01-1 R-19129	not valid
Poecilus cupreus	8.0 g as/ha in winter wheat (after harvest) No significant effects on mortality and feeding rate	KIIIA1 10.5.3/01 SXR/HF 63 Schmuck, 1993 M-052352-01-1 R-19131	valid
Carbid beetles (dominant field species: *Pterostichus madidus, Loricera pilicornis*)	8.0 g as/ha winter wheat (stadium with ear) No significant effects on mortality and feeding rate	KIIIA1 10.5.3/04 SXR/HF 56 Schmuck, 1992 M-052680-01-1 R-19138	additional information

B.9.5.2.1 Standard laboratory studies with non-target arthropods

KIIIA1 10.5.1/01 (newly submitted with the dossier)

Author:	Roig, J.
Title:	A Tier 1 laboratory dose-response study to assess the LR50 of Bulldock 25 EC for the parasitoid wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae) in ventilated glass cages
Date:	3 March 2014
Doc ID:	M-479582-01-1
Report no.:	FC011ARL
Edition no.:	R-33355
Guidelines:	A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae) (Mead-Briggs et al., 2000)
GLP:	yes
Validity:	valid for mortality, no determination of reproductive effects

Executive Summary
Bulldock 25 EC was applied to the glass plates of *Aphidius* cages at five nominal rates, of 20, 40, 80, 160 and 320 mg as/ha, at a spray application volume of approximately 200 L/ha. The control was treated with de-ionised water. Dimethoate at a rate of 120 mg as/ha was used as a reference item. *Aphidius rhopalosiphi* (DeStephani-Perez) was exposed in groups of 10 per unit to dry residues within 1.5 hours after application. There were 4 units for the water control, 4 units for each test item rate and 4 units for the reference item.
Mortality of the water and test item treatments was assessed after a 2 hours, 1 day and 2 days of exposure.
All validity criteria were met. Therefore the test was valid for the purposes to which it was designed. Control mortality indicated that test animals were in good condition. Mortality in the toxic reference, showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.

After 2 days of exposure to the test item at rates equivalent to up to 40 mg as/ha, survival of *Aphidius rhopalosiphi* was not statistically significantly reduced compared to the water control. Exposure to rates equivalent to 80, 160 and 320 mg as/ha had a significant effect on survival. The LR_{50} was calculated as 163 mg as/ha with 95 % confidence limits of 119 and 252 mg as/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Test item: Bulldock 25 EC
Description: Emulsifiable concentrate
Lot/Batch #: 92110164
Purity/content (as): 24.79 g/L, 2.752 % (w/w) (analysed)

2. Vehicle and/or positive control: Dafene Progress EC 400 (dimethoate, 400 g/L)

3. Test organisms:
Species: *Aphidius rhopalosiphi* (DeStephani-Perez)
Age: adults

Source: Katz Biotech AG, Germany
Diet/Food: Sugar solution: Bee Fit® HM

4. Environmental conditions:
Temperature: 20.1±0.7 °C
Photoperiod: 16 hours light (100 – 2000 lux): 8 hours dark
Humidity : 56.5±6.0 %

B. STUDY DESIGN

1. Experimental treatments
Bulldock 25 EC was applied to both inner sides of the glass plates that confine the wasps in the units at five nominal rates, of 20, 40, 80, 160 and 320 mg as/ha, at a nominal spray application volume of 200 L/ha. The control was treated with de-ionised water. Dimethoate at a rate of 120 mg as/ha was used as reference item.
Aphidius rhopalosiphi (DeStephani-Perez) was exposed in groups of 10 per unit to dry residues within 1.5 hours after application. There were 4 units for the water control, 4 units for each test item treatment and 4 units for the reference item.

2. Observations
After a 2 hour, 1 day and 2 days exposure period, the condition of the test animals was recorded as follows: alive (active), affected (slow movements, reduced coordination or any other abnormal behavior), moribund (unable to walk, but still moving antennae or legs), dead (motionless, abnormal posture).

3. Statistical calculations
Statistical analysis tested the null hypothesis that parameter values for the control group and the groups treated with test item were obtained from the same population. The null hypothesis was rejected if the probability of observing the test statistic (type I error level) fell below 5 % (alpha = 0.05).
Mortality was analysed by pair wise comparison to the water-treated control with Fisher's Exact Test (one sided).
Given the replication and the control mortality of the test, the power of the analysis was 90 % to detect an effect size of 25 % (McIndoe, 1997).
The relationship between corrected mortality and application rate, as well as the calculation of LR_{50}-values was established through Probit analysis. In addition all data were analysed graphically using Box and Whisker plots. SPSS Statistics 21 for Windows was used for statistical analyses and Probit regression.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Average escape rate (number of animals not retrieved over the initial number) was 1.4 % for the whole test. No animals escaped during the exposure period, except for 4 animals from one unit of the lowest test item rate, which 2 days after the application went missing. Since on the assessment 1 day after application these animals were present and alive, their disappearance was considered an artifact due to handling, rather than an effect of the test item. It was therefore decided to subtract 4 animals from the initial number entered instead of computing them as dead.
All validity criteria (for determination of mortality) were met. Therefore the test was valid for the purposes to which it was designed.

Table B.9.5-12: Validity criteria

	Validity criterion	Finding	Valid / not valid
Mortality de-ionised water control	≤ 15 %	0 %	Valid
Corrected mortality toxic reference	50 – 100 %	100 %	Valid

The sex of the animals was determined 2 days after the exposure period. In average more than 5 females per treatment were identified. However 2 units, 1 in the highest rate and 1 in the reference item treatment contained 4 females each. In addition the unit with 4 escapees had 2 females.

Mortality
Approximately 2 hours after the application some wasps from the 160 and 320 mg as/ha rate appeared to be affected and a few in the highest rate moribund. One day after start of exposure the number of affected wasps increased in the 40, 80 and 160 mg as/ha rates with 4, 7 and 7 affected wasps respectively.
However a large proportion of these had recovered on day 2.

On day 2 *Aphidius rhopalosiphi* exposed to the units treated with the test item showed a clear dose-related response. Mortality in the 2 lowest rates was not statistically significant compared to the de-ionised water treated units (Fisher's Exact Test, one-sided α=0.05). The observed mortality from these rates (20 and 40 mg as/ha) was 3 % (P=0.494) and 5 % (P=0.247) respectively. The calculated effects at the higher rates were statistically significant compared to the water control. The observed mortality at 80 mg as/ha was 25 % (P=0.001), at 160 mg as/ha was 29 % (P<0.001) and at 320 mg as/ha was 90 % (P<0.001).
The observed mortality in the reference item treated units was 100 % after a 1-day exposure.

Table B.9.5-13: Mortality of *Aphidius rhopalosiphi* after a 2-day exposure to Bulldock 25 EC

Test Item	**Bulldock 25 EC**	
Test organism	***Aphidius rhopalosiphi***	
Nominal application volume	**200 L/ha**	
Exposure	**2 days**	
	Mortality after 2 days	
De-ionised water control	0 %	
Application rates (mg as/ha):		
20	3 %	P=0.494
40	5 %	P=0.247
80	25 %	P=0.001*
160	29 %	P<0.001*
320	90 %	P<0.001*
Reference item	100 %	P<0.001*
LR_{50} (95 % confidence limits)	163 mg as/ha (119 and 252 mg as/ha)	
NOEC	40 mg as/ha	

*Statistically significantly different from de-ionised water control. Statistical analysis with Fisher's Exact Test, onesided.

Zero control mortality indicated that test animals were in good condition. Mortality in the reference item, showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.

III. CONCLUSIONS

After 2 days of exposure to the test item at rates equivalent to up to 40 mg as/ha, survival of *Aphidius*

rhopalosiphi was not statistically significantly reduced compared to the water control. Exposure to rates equivalent to 80, 160 and 320 mg as/ha had a significant effect on survival. The LR_{50} was calculated as 163 mg as/ha with 95 % confidence limits of 119 and 252 mg as/ha.

As no effects on reproduction were determined, it is not possible to derive an ER_{50}.
Therefore, the study is regarded as incomplete.

KIIIA1 10.5.1/02 (newly submitted with the dossier)

Author:	Roig, J.
Title:	A Tier 1 laboratory dose-response study to assess the LR50 of Bulldock 25 EC for the evaluate the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) in ventilated glass cages
Date:	3 March 2014
Doc ID:	M-479587-02-1
Report no.:	FC010TPL
Edition no.:	R-33356
Guidelines:	Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products (Blümel et al., 2000)
GLP:	yes
Validity:	valid for mortality, no determination of reproductive effects

Deviations: none

Executive Summary

Bulldock 25 EC was applied to glass and inert PTFE mortality units (‘coffin cells’) at five nominal rates, of 0.3, 0.9, 2.7, 8.1 and 24.3 mg as/ha, at a spray application volume of approximately 200 L/ha. The control was treated with deionised water. Dimethoate at a rate of 4.4 g as/ha was used as a reference item.
Typhlodromus pyri Scheuten was exposed in groups of 12 per unit to dry residues within 1.5 hours after application. There were 8 units for the water control, 5 units for each Bulldock 25 EC treatment and 4 units for the reference item.
Mortality of the water and test item treatments was assessed after a 7-day exposure period. The toxic reference treatment was assessed 4 days after the application.
All validity criteria were met. Therefore the test was valid for the purposes to which it was designed.

Low mortality in the control treatment indicated that test animals were in good condition. Mortality in the reference item, showed that test animals were sufficiently sensitive and that potential mortality effects of exposure to test item residues could be detected with the set-up used in this experiment.

After 7 days of exposure to Bulldock 25 EC at a rate equivalent to 0.3 mg as/ha, survival of *Typhlodromus pyri* was not statistically significantly reduced compared to the water control. Exposure to rates equivalent to 0.9, 2.7, 8.1 and 24.3 mg as/ha had a significant effect on survival. The LR_{50} was calculated as 2.50 mg as/ha with 95 % confidence limits of 1.62 and 3.38 mg as/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Bulldock 25 EC
Description: Emulsifiable concentrate

Lot/Batch #:	92110164
Purity/content (as):	24.79 g/L, 2.752 % (w/w) (analysed)
2. Vehicle and/or positive control:	Dafene Progress EC 400 (dimethoate, 400 g/L)
3. Test organisms:	
Species:	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Age:	1-day old protonymphs
Source:	PK Nuetzlingszuchten AG, Germany
Diet/Food:	Apple pollen
4. Environmental conditions:	
Temperature:	24.5±0.8 °C
Photoperiod:	16 hours light (100 – 2000 lux): 8 hours dark
Humidity:	62.2±5.2 %

B. STUDY DESIGN

1. Experimental treatments
Bulldock 25 EC was applied to all parts of the test units in contact with the mites (top and bottom glass plates, inert PTFE material between the glass plates and the connections for ventilation) at five nominal rates of0.3, 0.9, 2.7, 8.1 and 24.3 mg as/ha, at a nominal spray application volume of 200 L/ha. The control was treated with deionised water. Dimethoate at a rate of 4.4 g as/ha was used as reference item.
Typhlodromus pyri Scheuten was exposed in groups of 12 per unit to dry residues within 1.5 hours after application. There were initially 8 units for the water control, 5 units for each test item treatment and 4 units for the reference item. However due to a technical problem with the ventilation system, water was sucked inside units via the water tray. This caused flooding of 1 water unit treatment and two 0.3 mg as/ha units. These units were excluded from analysis. In addition probably a third 0.3 mg as/ha unit was also flooded during the exposure phase but this could not be confirmed.

2. Observations
Four days after initiation of the bioassay the set-up was inspected and food and water were added. Mortality was assessed 7 days after initiation (4 days for the reference item treated units) by recording the number of live males/females/juveniles and the number of corpses. In addition, the number of eggs was recorded.

3. Statistical calculations
Statistical analysis tested the null hypothesis that parameter values for the control group and the groups treated with test item were obtained from the same population. The null hypothesis was rejected if the probability of observing the test statistic (type I error level) falls below 5 % (alpha = 0.05).
Mortality data were analysed with Fisher's Exact Test.
Given the replication and the control mortality of the test, the power of the analysis was >80 % to detect an effect size of 25 % (McIndoe, 1997).
The relationship between corrected mortality and application rate, as well as the calculation of LR50-values was established through Probit analysis. In addition all data were analysed graphically using Box and Whisker plots.
SPSS Statistics 21 was used for statistical analyses and Probit regression.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Average escape rate (number of animals not retrieved over the initial number) was 11 % for the whole test.

Escaped mites were analysed as dead.
All validity criteria (for determination of mortality) were met. Therefore the test was valid for the purposes to which it was designed.

Table B.9.5-14: Validity criteria

	Validity criterion	Finding	Valid / not valid
Mortality de-ionised water control	≤ 20 %	15 %	Valid
Corrected mortality toxic reference	50 – 100 %	100 %	Valid

Mortality
T. pyri exposed to the test item treated units showed a well-defined dose response 7 days after the application. The lowest rate (0.3 mg as/ha) showed a mortality of 20 %. This effect was not statistically significant compared to the deionised water treated units (P=0.115, Fisher's Exact Test, α=0.05). The observed mortality from units treated at 0.9 mg as/ha was 28 %, at 2.7 mg as/ha was 41 %, at 8.1 mg as/ha was 82 % and at 24.3 mg as/ha was 100 %. The mortality of all tested rates above 0.3 mg as/ha was statistically significant compared to the water control (P<0.001). The observed mortality in the reference item treated units was 100 % after 4 days.

In the water control, 99 % of all surviving individuals had developed into adults on day 7. In the test item treatment group, however, delayed development (measured as the % juveniles of surviving individuals on day 7) was observed. For the 0.3, 0.9, 2.7 and 8.1 mg as/ha units, 13 %, 5 %, 5 % and 22 % respectively of the surviving mites had developed into adults.

Table B.9.5-15: Mortality of *Typhlodromus pyri* after a 7-day exposure to Bulldock 25 EC

Test Item	Bulldock 25 EC	
Test organism	*Typhlodromus pyri*	
Nominal application volume	200 L/ha	
Exposure	7 days on glass and inert PTFE mortality units (Coffin cells)	
	Mortality after 7 days	
De-ionised water control	15 %	
Application rates (mg as/ha):		
0.3	20 %	P=0.115
0.9	28 %	P=0.004*
2.7	41 %	P<0.001*
8.1	82 %	P<0.001*
24.3	100 %	P<0.001*
Reference item	100 %	P<0.001*
LR50 (95 % confidence limits)	2.50 mg as/ha (1.62 and 3.38 mg as/ha)	
NOEC	0.3 mg as/ha	

*Statistically significantly different from deionised water control. Statistical analysis with Fisher's Exact Test

Low mortality in the control treatment indicated that test animals were in good condition. Mortality in the toxic reference, showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.

III. CONCLUSIONS

After 7 days of exposure to Bulldock 25 EC at a rate equivalent to 0.3 mg as/ha, survival of *Typhlodromus pyri* was not statistically significantly reduced compared to the water control. Exposure to rates equivalent to 0.9, 2.7, 8.1 and 24.3 mg as/ha had a significant effect on survival. The LR_{50} was calculated as 2.50 mg as/ha with 95% confidence limits of 1.62 and 3.38 mg as/ha.

As no effects on reproduction were determined, it is not possible to derive a ER_{50}. Therefore, the study is incomplete.

KIIIA1 10.5.1/03

Author:	Heimbach, F.
Title:	Toxicity of Bulldock (025 EC) to carabid beetles (*Poecilus cupreus*)
Date:	30 June 1990
Doc ID:	M-052707-01-1
Report no.:	HBF/CA 27
Edition no.:	R-19124
Guidelines:	Test-Proposal Biologische Bundesanstalt für Land- und Forstwirtschaft, D – 3300 Braunschweig November 1989
GLP:	yes
Validity:	valid

Deviations: The study meets requirements from Heimbach *et al*., 2000 guideline

Test material: Beta-cyfluthrin 025 EC (Bulldock), as content: 25.6 g/L, formulation no. 103 according to 04112/0004

Results: The data of this study indicate that Bulldock 025 EC (beta-cyfluthrin) used under practical conditions in the field at the dosage of 300 mL/ha (7.7 g as/ha) should have no influence on carabid beetles as represented by *Poecilus cupreus*. The exposure under the chosen experimental conditions is even more stringent than under field conditions.

Conclusion: EC_{50} > 7.7 g as/ha

KIIIA1 10.5.1./04

Author:	Schmuck, R.
Title:	Effects of Bulldock EC 025 on the life cycle of rove beetles (Aleochara bilineata) under laboratory conditions
Date:	5 December 1992
Doc ID:	
Report no.:	SXR/AL 11
Edition no.:	M-052616-01-1 (R-19128)
Guidelines:	IOBC/WPRS Guideline 5.2.5
GLP:	yes
Validity:	not valid

Deviations: not valid

Test material: Beta-cyfluthrin EC 025 (Bulldock), as content: 27.3 g/L, formulation no. 134 according to 04112/0004.

Results:
The results show that under actual farming conditions minor effects on rove beetles, asrepresented by

Aleochara bilineata, are anticipated from an application.

Conclusion:
The study is not valid according the current guideline (Grimm et al.; IOBC Guideline f. non-target arthropods, 2000).
1. Natural soil with an unkown content of organic carbon was used.
2. According the study report the detection of mortality was uncertain due to difficulties to find the dark beetles on the dark soil. It was speculated about a possible higher mortality rate.
3. The mortality rate of the positive control was only 25.7 % instead of ≥ 50 %

Therefore, results can not be used for assessing the risk to NTAs.

KIIIA1 10.5.1./05

Author:	Kuehner, C.
Title:	Erfassung der Nebenwirkungen von BAY 13210 I auf die Florfliege, *Chrysoperla carnea* Steph. im Labor GAB Biotechnologie GmbH, Niefern-Oeschelbronn, Germany
Date:	16 March 1993
Doc ID:	M-052746-01-2
Report no.:	92022/01-CC
Edition no.:	R-19127
Guidelines:	Bigler (1988)
GLP:	yes
Validity:	not valid

Deviations: The study meets requirements from Bigler et al. guideline, but at the test item rate mortality was 100 % and no toxic reference was tested

Test material: Beta-cyfluthrin 125 SC (Bulldock), as content: 121 g/L, batch no. 233125837

Results: None out of 45 lacewing larvae which were exposed to spray deposits of 9.1 g as/ha (0.075 Lproduct/ha, tested with beta-Cyfluthrin 125 SC) on glass plates could successfully complete the metamorphosis.

Conclusion:
As mortality was 100 % at the test item rate, no endpoint can be derived.
Due to the lack of a positive control the test is not valid.

B.9.5.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

KIIIA1 10.5.2./01

Author:	Kleiner, R.
Title:	Testing toxicity to beneficial arthropods cereal aphid parasitoid - Aphidius rhopalosiphi (Destefani - Perez) / pupae according to IOBC guideline (Mead-Briggs 1992)
Date:	13 January 1997
Doc ID:	
Report no.:	97 10 48 004

Edition no.:	M-052305-01-1 (R-19126)
Guidelines:	IOBC Guideline (Mead-Briggs, 1992)
GLP:	yes
Validity:	-

Deviations: The study meets requirements from Mead-Briggs *et al*., 2000 guideline but mortality in test rate and toxic reference was 100 %
Test material: Beta-cyfluthrin (FCR 4545) 025 SC, as content: 25.08 g/L, development no. 0159177
Results: A 10-day exposure of the pupae to spray deposits of beta-Cyfluthrin (FCR 4545) 025 SC applied at 1.67 g as/ha (= 70 g/ha) resulted in 100 % mortality of the exposed pupae. Therefore, the parasitisation efficacy of the exposed wasps could not be tested.

Conclusion: no EC_{50}/LC_{50} derivable

KIIIA1 10.5.2/02

Author:	Neumann, P
Title:	Acute effects of Beta-cyfluthrin EC 025 on larvae of carabid beetles (Poecilus cupreus) under extended laboratory test conditions
Date:	2001
Doc ID:	M-080415-01-1
Report no.:	NNP/PC005
Edition no.:	E371 1666-0
Guidelines:	Internal testing method, Bayer AG
GLP:	yes
Validity:	n.a. (no official guidline), plausible

Material and methods:

The effect of Beta-cyfluthrin EC025 (content of as: 24.8 g/L beta-cyfluthrin, TOX No.: 4740-00, specification: article No.: 04000234, batch No.: 233825173) on larvae of *Poecilus cupreus* was assessed under extended laboratory conditions. Twenty larvae per treatment (1 per test cup) were exposed to soil residues of the test substance. The soil substrate (Lufa 2.1) was moistened to about 40 % of its water holding capacity. Test application rates (nominal) were 0.04, 0.40 and 4.0 mg as/kg soil (dry weight), respectively. The whole study duration was 65 days. Endpoints were mortality (individuals, which fail to hatch successfully), development time (time to metamorphosis) and adult body weight.

Findings:

Test species	*Poecilus cupreus* (larval stages)			
Exposure	natural standard soil (Lufa 2.1)			
Test formulation	Control	Beta-cyfluthrin EC 025		
Application rate	-	0.04 mg as/kg	0.40 mg as/kg	4.00 mg as/kg
Mortality rate [%]	10	0	100*	100*
Time to meta-morphosis [d]	41.0	49.1*	-	-
Mean adult body mass [mg]	80.3	73.7*	-	-
Effect on body mass [%]	-	8.2	-	-

*statistically significant different from the control treatment ($p < 0.05$).

LR_{50}> 0.04 mg as/kg soil
$LR_{100} \leq$ 0.4 mg as/kg soil
NOEC < 0.04 mg/as kg/soil
The reference treatment (850 mg Curaterr 5 GR/ test container) caused a mortality rate of 100 %.

Conclusion:
An exposure to beta-cyfluthrin at 0.40 and at 4.0 mg as/kg resulted in a mortality of 100 %. The mortality at 0.04 mg as/kg was not increased but the time to metamorphosis was significantly prolonged and the mean adult body mass was significantly reduced in statistical comparison to the control treatment.

KIIIA1 10.5.2./06

Author:	Aldershof, S.A
Title:	Bulldock EC025: An extended laboratory dose-response study to evaluate the effects on the parasitoid *Aphidius rhopalosiphi* on detached apple leaves
Date:	13 August 1999
Doc ID:	M-015321-01-1
Report no.:	B042ARE
Edition no.:	R-19122
Guidelines:	Based on Mead-Briggs, 1992, Polgar, 1988, SETAC/ESCORT (1994)
GLP:	yes
Validity:	not reliable

Deviations: not plausible/reliable

Test material: Beta-cyfluthrin EC025 (Bulldock), as content: 24.8 g/L, batch no. 233825173

Results:
The LC50 of Bulldock EC025 to the parasitoid wasp *Aphidius rhopalosiphi*, based on mortality due to adult exposure to residues applied to apple leaves in ventilated cages, is equivalent to 17 g as/ha. Concentrations of Bulldock EC025 of 5.4 g as/ha or higher caused mortality that was statistically higher than control mortality. Bulldock EC025 1.7 g as/ha and 5.4 g as/ha were identified as NOEC and LOEC, respectively.
Reproduction at the lowest 3 test concentrations of Bulldock EC025; 0.2, 0.7 and 1.7 g as/ha, was not statistically different from control performance. Hence, Bulldock EC025 1.7 g as/ha can be regarded as NOEC regarding mortality as well as fecundity.

Conclusion:

The study is not appropriate to derive a meaningful and reliably LR/ER50. Thus, it is not plausible.
Extended 2-D laboratory tests should be leant on the corresponding guidlines for laboratory tests (Bigler et al.; IOBC Guideline f. non-target arthropods, 2000). Both tests should only differ in the used substrate (glass plates vs. natural substrate – e.g. apple leaves).
Due to the test arrangement a sufficient, comparable exposure of the test organisms cannot be warranted. Moreover leaves were not sprayed with a 10 % ual sucrose solution.
The insuffient and erratic exposure resulted in high variabilities of mortality rates:
Treatment group 17 g ai/ha : 31,25 % - 80 % (mean = 53.3 %, standard deviation = 24.4 %)
Treatment group 1.7 g ai/ha : 6.25 % - 40 % (mean = 22.08 %, standard deviation = 16.97 %)
Further more, 3 replicates were excluded [“escaping rate” > 20 % (3/15)] due to internal rules of the testing institute and therefore, no data were given.

KIIIA1 10.5.2/03

Author:	Aldershof, S.A.
Title:	Bulldock EC025: An extended laboratory dose-response study to evaluate the effects on the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) on detached apple leaves
Date:	15 July 1999
Doc ID:	M-022573-01-1
Report no.:	B043TPE
Edition no.:	R-19120
Guidelines:	Based on Blümel *et al.*, ringtest guideline in prep, Bakker *et al.* (1992), EPPO Guideline 142 (1989) SETAC/ESCORT (1994)
GLP:	yes
Validity:	not valid

Deviations: not valid

Test material: Beta-cyfluthrin EC 25 (Bulldock), as content: 24.8 g/L, batch no. 233825173

Results: The LC_{50} of Bulldock EC 25 to the predatory mite *Typhlodromus pyri*, based on mortality due to juvenile exposure to residues in ventilated Munger cages, is equivalent to 0.24 g as/ha. All concentrations of Bulldock EC 25 caused mortality that was statistically higher than control mortality. Therefore, no NOEC or LOEC could be identified.
Reproduction at the lowest two test concentrations of Bulldock EC 25, 0.1 and 0.2 g as/ha, was reduced with 25 % and 45 %, respectively, compared to control performance. These oviposition rates were above or close to the validity threshold of 4 eggs per female, and did not differ statistically from control performance. Oviposition in the Bulldock EC 25 0.3 g as/ha rate was significantly reduced compared to the water control with 78 %.

Conclusion:
The study is not appropriate to derive a meaningful and reliably LR/ER_{50}. Thus, it is not plausible.
1. Test unit was partly open (an open hole). Looking at the table "Mortality Dose Response test“, it becomes obvious that animals were able to escape from the test unit and to return later.
2. According to the study report apple leaves were strongly corrugated after 7 days. Thus, animals could hide below the leaves and avoid exposure.
As mites could avoid the contact with the test substance, the real exposure is not assessable.
3. The mortality rate of the positive control was only 15 %. In the study report this was reasoned with an erroneously wrong concentration of the reference substance.

KIIIA1 10.5.2./04

Author:	Kleiner, R.
Title:	Beta-Cyfluthvrin EC025 - Toxicity to larvae of *Coccinella septempunctata* l. under extended laboratory conditions
Date:	16 March 1993
Doc ID:	
Report no.:	99 10 48 120
Edition no.:	M-032166-01-1 (R-19125)

Guidelines:	BBA Guideline VI, 23-2.1.5 (1989), IOBC Guideline proposal (Schmuck *et al.*, 1999)
GLP:	yes
Validity:	not valid

Deviations: not valid

Test material: 1st run: Beta-cyfluthrin EC 025, as content: 24.8 %, batch no. 233825173
2nd run: Beta-cyfluthrin EC 025, as content: 25.4 %, batch no. 233925628

Results: Based on the mortality results of the 2nd run the LR_{50} of beta-cyfluthrin EC 025 was 0.0261 g as/ha (95 % confidence intervals: 0.0148 g as/ha and 0.0461 g as/ha).
No statistically significant differences in reproduction were observed in any of the beta-cyfluthrin EC025 treatment groups when compared to the control group.

Conclusion:
The study is not appropriate to derive a meaningful and reliable LR/ER_{50}. Thus, it is not plausible.

Extended 2-D laboratory tests should be leant on the corresponding guidlines for laboratory tests (Bigler et al.; IOBC Guideline f. non-target arthropods, 2000). Both tests should only differ in the used substrate (glass plates vs. Natural substrate – e.g. apple leaves). Accordingly, 100 % of the ground area must be covered with natural substrate (apple leaves). Due to the test arrangement (one leave on a Petri dish of 9 cm diameter) a sufficient, comparable exposure of the test organisms cannot be warranted.

KIIIA1 10.5.2./05

Author:	Neumann, P.
Title:	Effect of Beta-cyfluthrin EC025 on the Ladybird Beetle (Coccinella septempunctata) under extended laboratory conditions (aged residue test)
Date:	5 June 2000
Doc ID:	M-038111-01-1
Report no.:	NNP/CS004
Edition no.:	R-19205
Guidelines:	BBA Guideline VI, 23-2.1.5 (1989), modified for an aged residue test; the reproduction part based on the most recent version of the IOBC ring testing group
GLP:	yes
Validity:	valid

Deviations: The study meets requirements from Schmuck *et al*., 2000 guideline

Test material: Beta-cyfluthrin EC 25, as content: 24.8 %, batch no. 233825173

Results: An application rate of 11.25 g as/ha caused mortality of 100 %, 28 % and 0 % after 0 days, 3 weeks and 7 weeks, respectively. The effect on reproduction was 48 % and 5 % after 3 weeks and 7 weeks, respectively.

KIIIA1 10.5.2./07 (newly submitted with the dossier)

Author:	Moll, M.
Title:	Effect of Beta-cyfluthrin EC025 on the Ladybird Beetle (Coccinella septempunctata) under extended laboratory conditions (aged residue test)
Date:	15 January 2004 (2004a)
Doc ID:	
Report no.:	18121013
Edition no.:	R-19424
Guidelines:	Schmuck *et al.* 2000: A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The Guideline was modified for exposure of *C. septempunctata* on natural substrate.
GLP:	yes
Validity:	valid

Deviations: none

Executive Summary
The effect of freshly dried and aged residues of Bulldock EC 25 (active ingredient: 25.5 g beta-cyfluthrin/L) on the pre-imaginal survival and reproduction of the ladybird beetle *Coccinella septempunctata* was tested in an extended laboratory study. The test item was applied two times in an interval of 14 days at 0.3 and 1.2 g as in 400 L water/ha in the field on potted bean plants. One larva per replicate (40 replicates per treatment group) was exposed to the spray residues on bean leaves compared to a water treated control and to a toxic standard. Additionally, an assessment for sublethal effects on reproduction of the survivors was made.
The 1st bioassay was carried out with freshly dried residues. Further bioassays on aged residues were started on day 3, 7 and 14 after the 2nd application.
All validity criteria according to the guideline were fulfilled.

Overall, effects on survival were less than 50 % after 14 days of aging for 2 applications at 0.3 or 1.2 g as/ha. Reproduction was not affected at 0.3 g as/ha. The low reproductive output per female at 1.2 g as/ha was unlikely to be directly related to treatment.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock EC 025 (beta-cyfluthrin)
Description:	Yellow to brown liquid
Lot/Batch #:	PF90034968
Purity:	25.5 g/L

2. Vehicle and/or positive control: Control: 400 L tap water/ha Positive control: Perfekthion (BAS 152 11I): 400 g/L dimethoate

3. Test organisms:

Species:	*Coccinella septempunctata*
Age:	Approximately 3 - 4 day old larvae
Source:	Katz Biotech AG, D-73642 Welzheim

Diet/Food:	Larvae: live aphids (*Acyrthosiphon pisum*, *Megoura viciae*) *ad libitum* Adults: live aphids (broad bean plant (*Vicia fabe*) infested with aphids *Acyrthosiphon pisum*, *Megoura viciae*) fine ground pollen and honey *ad libitum*

4. Environmental conditions:

Temperature:	23 - 27 °C
Humidity:	60 - 87 %
Light intensity:	1080 – 2800 lux
Photoperiod:	16 hours light : 8 hours dark

B: STUDY DESIGN AND METHODS

1. **Experimental treatments**

The test item was applied two times in an interval of 14 days at 0.3 and 1.2 g as in 400 L water/ha in the field on potted bean plants. Application and aging of the test item was done in the field under natural conditions. After the applications leaves cut from the treated bean plants were used for the bioassays.

2. Observations

Number of living and dead larvae and pupae and number of adults hatched was counted daily. Mortality of the adults was assessed daily and the sex of the dead beetles was determined. Number of eggs was counted daily within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality was ≤ 50 %.

3. Statistical calculations

Mortality data were analyse for significance using the Fisher Exact Test, which is a distribution free test and does not require testing for normality or homogeneity prior to analysis. Reproduction data were tested for normal distribution using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$). In the 4th bioassay, because reproduction data (larval hatching rate) were normally distributed and homogeneous the Dunnett-Test (multiple comparison, two-sided, $\alpha = 0.05$) was used. Because reproduction data (eggs per female per day) were normally distributed and not homogeneous the Bonferoni-t-test (multiple comparison, two-sided, $\alpha = 0.05$) was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

The toxic standard treatment resulted in 94.3 % corrected mortality, indicating the suitability of the test procedure. Biological results of the Bulldock EC 25 and control treatment are summarised below.

Table B.9.5-16: Effects of Bulldock 25 EC on pre-imaginal mortality of *C. Septempunctata*

Parameter measured	Treatment group		
	Control	Bulldock 25 EC	
		2 x 0.3 g as/ha	2 x 1.2 g as/ha
Day of 2nd application Mortalitya (%) Corrected mortality (%)	12.5 -	92.5* 91.4	100.0* 100.0
3 days after 2nd application Mortality[a] (%) Corrected mortality (%)	22.5 -	95.0* 93.5	100.0* 100.0
7 days after 2nd application Mortality[a] (%) Corrected mortality (%)	25.0 -	90.0* 86.7	100.0* 100.0

14 days after 2nd application Mortality[a] (%) Corrected mortality (%)	10.0 -	20.0 (n.s.) 11.1	27.5 19.4

a 40 individuals per treatment group, exposure on leaves from treated bean plants
* Significant
n.s. Not significant

Table B.9.5-17: Effects of Bulldock 25 EC on the development and reproduction of *C. septempunctata* (14 day aged residues)

Parameter measured	Treatment group[a]		
	Control	Bulldock 25 EC	
		2 x 0.3 g as/ha	2 x 1.2 g as/ha
Number of eggs /female/day[b]	5.5 ± 3.3	12.8 ± 6.8*	1.5 ± 1.5*
Number of fertile eggs/female/day	4.8 ± 3.4	10.5 ± 6.0 *	1.0 ± 0.9*
Larval hatching rate (%)	85.8 ± 15.4	80.0 ± 18.1 (n.s.)	66.3 ± 17.6*

a adults developed from larvae exposed to spray residues on bean leaves
b Oviposition started 1 week after the first egglaying was observed in the control and lasted 2 weeks
* Significant
n.s. Not significant

All validity criteria of the study were met. The control mortality was below 30 % (10.0 – 25.0 %), the mortality in the toxic standard was >40 % (94.3 %) and the control reproduction rate was ≥ 2 fertile eggs per viable female per day (4.8).

III. CONCLUSIONS

Pre-imaginal survival of *Coccinella septempunctata* was statistically significantly affected compared to the control by exposure to freshly dried residues (day of 2nd application) and aged residues of Bulldock EC 25 up to and including 7 days after 2nd application with an application rate of 0.3 and 1.2 g as/ha.
For 14 days aged residues there was no statistically significantly effect on survival for 0.3 and 1.2 g as/ha of Bulldock EC 25 compared to the control (corrected mortality 11.1 and 19.4 %).
The reproductive capacity of adult *C. septempunctata* was assessed in the bioassay started 14 days after 2nd application for the control and both tested treatment groups of Bulldock EC 25 (0.3 and 1.2 g as/ha. Reproduction of the control and the test item treatment group 0.3 g as/ha was > 2 fertile eggs per viable female per day in both bioassays, so the reproductive output is within the historical data base for control beetles. Therefore, this parameter is considered as not effected by the treatment of 0.3 g as/ha (Schmuck et al. 2000). At 1.2 g as/ha the number of fertile eggs was below 2 fertile eggs per viable female per day (1.0 fertile eggs per viable female per day). The sex ratio of adults in the 1.2 g as/ha group at the start of this reproduction assay was 1 male : 4 females compared with around 1 : 1 in the control. The imbalance between males and females is likely to have affected the reproductive output per female, rather than a direct influence of the test material.
Since there were no effects of Bulldock EC 25 on pre-imaginal mortality and reproduction of *Coccinella septempunctata* 14 days after the 2nd application, further testing with aged residues was not necessary.
Overall, effects on survival were less than 50 % after 14 days of aging for 2 applications at 0.3 or 1.2 g as/ha. Reproduction was not affected at 0.3 g as/ha. The low reproductive output per female at 1.2 g as/ha was unlikely to be directly related to treatment.

KIIIA1 10.5.2./08 (newly submitted with the dossier)

Author:	Moll, M.
Title:	Effects of Bulldock EC 025 on the Ladybird Beetle *Coccinella septempuntata*, Extended Laboratory Study – Aged Residue Test – Field Application Rate

Date:	15 January 2004 (2004b)
Doc ID:	
Report no.:	18122013
Edition no.:	R-19425
Guidelines:	Schmuck *et al.* 2000: A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The Guideline was modified for exposure of *C. septempunctata* on natural substrate.
GLP:	yes
Validity:	valid

Deviations: none

Executive Summary
The effect of freshly dried and aged residues of Bulldock 25 EC (active ingredient: 25.5 g beta-cyfluthrin/L) on the pre-imaginal survival and reproduction of the ladybird beetle *Coccinella septempunctata* was tested in an extended laboratory study. The test item was applied two times in an interval of 14 days at 12.5 g as in 400 L water/ha in the field on potted bean plants. One larva per replicate (40 replicates per treatment group) was exposed to the spray residues on bean leaves compared to a water treated control and to a toxic standard. Additionally, an assessment for sublethal effects on reproduction of the survivors was made.
The 1st bioassay was carried out with freshly dried residues. Further bioassays on aged residues were started on day 14 and 28 after the 2nd application.

All validity criteria according to the guideline were fulfilled.

Overall, effects of aged residues of Bulldock EC 25 applied twice at 12.5 g as/ha were less than 50 % and reproduction was > 2 fertile eggs per viable female per day after treated plants were kept outdoors for 28 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock EC 025 (beta-cyfluthrin)
Description:	Yellow to brown liquid
Lot/Batch #:	PF90034968
Purity:	25.5 g/L
2. Vehicle and/or positive control:	Control: 400 L tap water/ha Positive control: Perfekthion (BAS 152 11I): 400 g/L dimethoate

3. Test organisms:

Species:	*Coccinella septempunctata*
Age:	Approximately 3 - 4 day old larvae
Source:	Katz Biotech AG, D-73642 Welzheim
Diet/Food:	Larvae: live aphids (*Acyrthosiphon pisum*, *Megoura viciae*) *ad libitum* Adults: live aphids (broad bean plant (*Vicia fabe*) infested with aphids *Acyrthosiphon pisum*, *Megoura viciae*) fine ground pollen and honey ad libitum

4. Environmental conditions:

Temperature:	23 - 27 °C
Humidity:	60 - 87 %
Light intensity:	1050 – 3600 lux

Photoperiod: 16 hours light : 8 hours dark

B: STUDY DESIGN AND METHODS

1. Experimental treatments

The test item was applied two times in an interval of 14 days at 12.5 g as in 400 L water/ha in the field on potted bean plants. Application and aging of the test item was done in the field under natural conditions. After the applications leaves cut from the treated bean plants were used for the bioassays.

2. Observations

Number of living and dead larvae and pupae and number of adults hatched was counted daily. Mortality of the adults was assessed daily and the sex of the dead beetles was determined. Number of eggs was counted daily within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality was ≤ 50 %.

3. Statistical calculations

Mortality data were analyse for significance using the Fisher Exact Test, which is a distribution free test and does not require testing for normality or homogeneity prior to analysis. Reproduction data were tested for normal distribution using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$). In the 3rd bioassay, because reproduction data (eggs per female per day) were normally distributed and homogeneous the Student-t-test for homogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used. Because reproduction data (larval hatching rate) were normally distributed and not homogeneous the Student-t-test for inhomogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used.
Mortality data were analyse for significance using the Fisher Exact Test, which is a distribution free test and does not require testing for normality or homogeneity prior to analysis. Reproduction data were tested for normal distribution using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$). In the 3rd bioassay, because reproduction data (eggs per female per day) were normally distributed and homogeneous the Student-t-test for homogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used. Because reproduction data (larval hatching rate) were normally distributed and not homogeneous the Student-t-test for inhomogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

The toxic standard treatment resulted in 94.3 % corrected mortality, indicating the suitability of the test procedure. Biological results of the Bulldock EC 25 and control treatment are summarised below.

Table B.9.5-18: Effects of Bulldock 25 EC on pre-imaginal mortality of *Coccinella septempunctata* applied at a rate of 2 x 12.5 g as/ha

Parameter measured	Treatment group[a]	
	Control	Bulldock 25 EC 2 x 12.5 g as/ha
Day of 2nd application	12.5	100.0*
Mortality[a] (%)	-	100.0
Corrected mortality (%)		
14 days after 2nd application	10.0	72.5*
Mortality[a] (%)	-	69.4
Corrected mortality (%)		
28 days after 2nd application	0.0	30.0*
Mortality[a] (%)	-	30.0
Corrected mortality (%)		

[a] 40 individuals per treatment group, exposure on leaves from treated bean plants
* significant

Table B.9.5-19: Effects of Bulldock 25 EC on reproduction of *C. septempunctata* at a rate of 2 x 12.5 g as/ha (28 day aged residues)

Parameter measured	Treatment group[a]	
	Control	Bulldock 25 EC 2 x 12.5 g as/ha
Number of eggs /female/day[b]	7.9 ± 4.0	5.7 ± 4.1 (n.s.)
Number of fertile eggs/female/day	5.3 ± 3.6	4.7 ± 3.5 (n.s.)
Larval hatching rate (%)	63.4 ± 26.9	82.7 ± 8.0 *

a adults developed from larvae exposed to spray residues on bean leaves
b Oviposition started 1 week after the first egglaying was observed in the control and lasted 2 weeks
* Significant
n.s. not significant

All validity criteria of the study were met. The control mortality was below 30 % (0.0 – 12.5 %), the mortality in the toxic standard was >40 % (94.3 %) and the control reproduction rate was ≥ 2 fertile eggs per viable female per day (5.3).

III. CONCLUSIONS

Pre-imaginal survival of *Coccinella septempunctata* was statistically significantly affected compared to the control by exposure to freshly dried residues (day of 2nd application) of Bulldock EC 25 and aged residues 14 days after 2nd application with an application rate of 12.5 g as/ha.
For 28 days aged residues, mortality was statistically significantly higher than the control, but the corrected mortality was below the ESCORT 2 trigger of 50 % (30 %).
The reproductive capacity of adult *C. septempunctata* was assessed in the bioassay started 28 days after 2nd application. Reproduction of the control and the test item treatment group was > 2 fertile eggs per viable female per day in both bioassays, so the reproductive output is within the historical data base for control beetles. Therefore, this parameter is considered as not effected (Schmuck et al. 2000).
Overall, effects of aged residues of Bulldock EC 25 applied twice at 12.5 g as/ha were less than 50 % and reproduction was > 2 fertile eggs per viable female per day after treated plants were kept outdoors for 28 days.

KIIIA1 10.5.2./09 (newly submitted with the dossier)

Author:	Moll, M.
Title:	Effects of Bulldock EC 025 on the Ladybird Beetle *Coccinella septempunctata*, Extended Laboratory Study – Aged Residue Test
Date:	03 November 2005 (2005a)
Doc ID:	
Report no.:	25141013
Edition no.:	R-19594
Guidelines:	Schmuck *et al.* 2000: A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The Guideline was modified for exposure of *C. septempunctata* on natural substrate.
GLP:	yes
Validity:	valid

Deviations: none

Executive Summary
The effect of freshly dried and aged residues of Bulldock 25 EC (active ingredient: 25.2 g beta-cyfluthrin/L) on the pre-imaginal survival and reproduction of the ladybird beetle *Coccinella septempunctata* was tested in an extended laboratory study. The test item was applied two times in an interval of 14 days at 20 g as in 400 L water/ha in the field on potted bean plants. One larva per replicate (40 replicates per treatment group) was exposed to the spray residues on bean leaves compared to a water treated control and to a toxic standard. Additionally, an assessment for sublethal effects on reproduction of the survivors was made.
The 1st bioassay was carried out with freshly dried residues. Further bioassays on aged residues were started on day 28 and 42 after the 2nd application.
All validity criteria according to the guideline were fulfilled.

Overall, effects on survival of aged residues of Bulldock EC 25 applied twice at 20 g as/ha were less than 50 % and reproduction was > 2 fertile eggs per viable female per day after treated plants were kept outdoors for 28 and 42 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 025 EC (beta-cyfluthrin)
Description:	Clear, yellow liquid
Lot/Batch #:	60111178
Purity:	25.2 g/L
2. Vehicle and/or positive control:	Control: 400 L tap water/ha Positive control: Perfekthion (BAS 152 11I): 400 g/L dimethoate
3. Test organisms:	
Species:	*Coccinella septempunctata*
Age:	Approximately 4 day old larvae
Source:	Katz Biotech AG, D-15837 Baruth
Diet/Food:	Larvae: live aphids (*Acyrthosiphon pisum*, *Megoura viciae*) *ad libitum* Adults: live aphids (broad bean plant (*Vicia fabe*) infested with aphids *Acyrthosiphon pisum*, *Megoura viciae*) fine ground pollen and honey *ad libitum*

4. Environmental conditions:

Temperature:	23 - 27 °C
Humidity:	61 - 84 %
Light intensity:	1050 – 4100 lux
Photoperiod:	16 hours light : 8 hours dark

B: STUDY DESIGN AND METHODS

1. Experimental treatments
The test item was applied two times in an interval of 14 days at 20 g as in 400 L water/ha in the field on potted bean plants. Application and aging of the test item was done in the field under natural conditions. After the applications leaves cut from the treated bean plants were used for the bioassays.

2. Observations
Number of living and dead larvae and pupae and number of adults hatched was counted daily. Mortality of the adults was assessed daily and the sex of the dead beetles was determined. Number of eggs was counted daily within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality was ≤ 50 %.

3. Statistical calculations

Mortality data were analyse for significance using the Fisher Exact Test, which is a distribution free test and does not require testing for normality or homogeneity prior to analysis. Reproduction data were tested for normal distribution using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous the Student-t-test for homogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used for the 2nd bioassay and for the 3rd bioassay for eggs per female per day. Because reproduction data were normally distributed and not homogeneous the Welch-t-test for inhomogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used in the 3rd bioassay for the larval hatching rate.

II. RESULTS AND DISCUSSION

A. FINDINGS

The toxic standard treatment resulted in 100 % mortality, indicating the suitability of the test procedure. Biological results of the Bulldock 25 EC and control treatment are summarised below.

Table B.9.5-20: Effects of Bulldock 25 EC on pre-imaginal mortality of *Coccinella septempunctata* applied at a rate of 2 x 20 g as/ha

Parameter measured	Treatment group[a]	
	Control	Bulldock 25 EC 2 x 20 g as/ha
Day of 2nd application Mortality[a] (%) Corrected mortality (%)	27.5	100.0* 100.0
14 days after 2nd application Mortality[a] (%) Corrected mortality (%)	2.5	47.5* 46.2
28 days after 2nd application Mortality[a] (%) Corrected mortality (%)	5.0	25* 21.1

a 40 individuals per treatment group, exposure on leaves from treated bean plants

Table B.9.5-21: Effects of Bulldock 25 EC on reproduction of *C. septempunctata* at a rate of 2 x 20 g as/ha (28 day aged residues)

Parameter measured	Treatment group[a]	
	Control	Bulldock 25 EC 2 x 20 g as/ha
Number of eggs /female/day[b]	12.7 ± 8.6	23.5 ± 12.0*
Number of fertile eggs/female/day	10.2 ± 7.3	19.0 ± 10.8*
Larval hatching rate (%)	77.0 ± 7.9	82.7 ± 8.0 (n.s.)

a adults developed from larvae exposed to spray residues on bean leaves
b Oviposition started 1 week after the first egglaying was observed in the control and lasted 2 weeks
* Significant
n.s. not significant

Table B.9.5-22: Effects of Bulldock 25 EC on reproduction of *C. septempunctata* at a rate of 2 x 20 g as/ha (42 day aged residues)

Parameter measured	Treatment group[a]	
	Control	Bulldock 25 EC 2 x 20 g as/ha
Number of eggs /female/day[b]	23.3 ± 7.9	16.3 ± 5.9 *

Number of fertile eggs/female/day	15.3 ± 5.1	12.5 ± 5.8 (n.s.)
Larval hatching rate (%)	66.1 ± 5.6	74.9 ± 13.2 *

[a] adults developed from larvae exposed to spray residues on bean leaves
[b] Oviposition started 1 week after the first egglaying was observed in the control and lasted 2 weeks
* Significant
n.s. not significant

All validity criteria of the study were met. The control mortality was below 30 % (2.5 – 27.5 %), the mortality in the toxic standard was >40 % (100 %) and the control reproduction rate was ≥ 2 fertile eggs per viable female per day (10.2 - 15.3).

III. CONCLUSIONS

Pre-imaginal survival of *Coccinella septempunctata* was statistically significantly affected compared to the control by exposure to freshly dried residues (day of 2nd application) of Bulldock EC 25 with an application rate of 20 g as/ha.
For 28 and 42 days aged residues, mortality was statistically significantly higher than the control, but the corrected mortality was below the ESCORT 2 trigger of 50 % (46.2 and 21.1 %).
The reproductive capacity of adult *C. septempunctata* was assessed in the bioassay started 28 and 42 days after 2nd application. Reproduction of the control and the test item treatment group was > 2 fertile eggs per viable female per day in both bioassays, so the reproductive output is within the historical data base for control beetles. Therefore, this parameter is considered as not effected (Schmuck et al. 2000).
Overall, effects on survival of aged residues of Bulldock EC 25 applied twice at 20 g as/ha were less than 50 % and reproduction was > 2 fertile eggs per viable female per day after treated plants were kept outdoors for 28 and 42 days.

KIIIA1 10.5.2./10 (newly submitted with the dossier)

Author:	Moll, M.
Title:	Effects of Baythroid 050 EC on the Ladybird Beetle *Coccinella septempunctata*, Extended Laboratory Study – Aged Residue Test
Date:	03 November 2005 (2005b)
Doc ID:	
Report no.:	25151013
Edition no.:	R-19593
Guidelines:	Schmuck *et al.* 2000: A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The Guideline was modified for exposure of *C. septempunctata* on natural substrate.
GLP:	yes
Validity:	valid

Deviations: none

Executive Summary

The effect of freshly dried and aged residues of Baythroid 050 EC (active ingredient: 49.4 g cyfluthrin/L) on the pre-imaginal survival and reproduction of the ladybird beetle *Coccinella septempunctata* was tested in an extended laboratory study. The test item was applied two times in an interval of 14 days at 40 g as in 400 L water/ha in the field on potted bean plants. One larva per replicate (40 replicates per treatment group) was exposed to the spray residues on bean leaves compared to a water treated control and to a toxic standard. Additionally, an assessment for sublethal effects on reproduction of the survivors was made.

The 1st bioassay was carried out with freshly dried residues. Further bioassays on aged residues were started on day 28 and 42 after the 2nd application.
All validity criteria according to the guideline were fulfilled.

Overall, effects on survival of aged residues of Baythroid 050 EC applied twice at 40 g as/ha were less than 50 % and reproduction was > 2 fertile eggs per viable female per day after treated plants were kept outdoors for 42 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Baythroid 050 EC (cyfluthrin)
Description:	Clear, yellow to brown liquid
Lot/Batch #:	050413
Purity:	49.4 g/L
2. Vehicle and/or positive control:	Control: 400 L tap water/ha Positive control: Perfekthion (BAS 152 11I): 400 g/L dimethoate
3. Test organisms:	
Species:	*Coccinella septempunctata*
Age:	Approximately 4 day old larvae
Source:	Katz Biotech AG, D-15837 Baruth
Diet/Food:	Larvae: live aphids (*Acyrthosiphon pisum*, *Megoura viciae*) *ad libitum* Adults: live aphids (broad bean plant (*Vicia fabe*) infested with aphids *Acyrthosiphon pisum*, *Megoura viciae*) fine ground pollen and honey *ad libitum*
4. Environmental conditions:	
Temperature:	23 - 27 °C
Humidity:	61 - 84 %
Light intensity:	1050 – 4100 lux
Photoperiod:	16 hours light : 8 hours dark

B: STUDY DESIGN AND METHODS

1. Experimental treatments
The test item was applied two times in an interval of 14 days at 40 g as in 400 L water/ha in the field on potted bean plants. Application and aging of the test item was done in the field under natural conditions. After the applications leaves cut from the treated bean plants were used for the bioassays.

2. Observations
Number of living and dead larvae and pupae and number of adults hatched was counted daily. Mortality of the adults was assessed daily and the sex of the dead beetles was determined. Number of eggs was counted daily within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality was ≤ 50 %.

3. Statistical calculations
Mortality data were analyse for significance using the Fisher Exact Test, which is a distribution free test and does not require testing for normality or homogeneity prior to analysis. Reproduction data were tested for normal distribution using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$).
Because reproduction data were normally distributed and homogeneous the Student-t-test for homogeneous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

The toxic standard treatment resulted in 100 % mortality, indicating the suitability of the test procedure. Biological results of the Baythroid 050 EC and control treatment are summarised below.

Table B.9.5-23: Effects of Baythroid 050 EC on pre-imaginal mortality of *Coccinella septempunctata* applied at a rate of 2 x 40 g as/ha

Parameter measured	Treatment group[a]	
	Control	Baythroid 050 EC 2 x 40 g as/ha
Day of 2nd application Mortality[a] (%) Corrected mortality (%)	27.5 -	100.0* 100.0
14 days after 2nd application Mortality[a] (%) Corrected mortality (%)	2.5 -	80.0* 79.5
28 days after 2nd application Mortality[a] (%) Corrected mortality (%)	5.0 -	37.5* 34.2

Table B.9.5-24: Effects of Baythroid 050 EC on reproduction of *C. septempunctata* at a rate of 2 x 40 g as/ha (42 day aged residues)

Parameter measured	Treatment group[a]	
	Control	Baythroid 050 EC 2 x 40 g as/ha
Number of eggs /female/day[b]	23.3 ± 7.9	18.9 ± 6.4 (n.s.)
Number of fertile eggs/female/day	15.3 ± 5.1	14.2 ± 5.8 (n.s.)
Larval hatching rate (%)	66.1 ± 5.6	74.0 ± 8.4*

a adults developed from larvae exposed to spray residues on bean leaves
b Oviposition started 1 week after the first egglaying was observed in the control and lasted 2 weeks
* Significant
n.s. Not significant

All validity criteria of the study were met. The control mortality was below 30 % (2.5 – 27.5 %), the mortality in the toxic standard was >40 % (100 %) and the control reproduction rate was ≥ 2 fertile eggs per viable female per day (15.3).

III. CONCLUSIONS

Pre-imaginal survival of *Coccinella septempunctata* was statistically significantly affected compared to the control by exposure to freshly dried residues (day of 2nd application) and aged residues (28 days after 2nd application) of Baythroid 050 EC with an application rate of 40 g as/ha.
For 42 days aged residues, mortality was statistically significantly higher than the control, but the corrected mortality was below the ESCORT 2 trigger of 50 % (34.2 %).

B.9.5.2.3 Semi-field studies with non-target arthropods

KIIIA1 10.5.3/01

Author:	Schmuck, R.
Title:	Acute effects of a spray application of Bulldock EC025 on carabid beetles (*Poecilus cupreus*) under semifield conditions
Date:	1 December 1993
Doc ID:	M-052352-01-1
Report no.:	SXR/HF 63
Edition no.:	R-19131
Guidelines:	The experiment followed the protocol established by the German registration authority (BBA)
GLP:	yes
Validity:	valid

Deviations: The study meets requirements from Heimbach et al., 2000 guideline

Test material: Beta-cyfluthrin EC 25 (Bulldock), as content: 26.6 g/L, batch no. 134 based on form. No. 04112/0004

Results: The results show that in a field situation (winter wheat after harvest), under normal management conditions, a spray application of Bulldock EC 25 applied at 300 mL/ha (8 g as/ha) poses no risk to carabid beetles, represented by *Poecilus cupreus*, active at ground level.

Conclusion: NOER = 8 g as/ha

KIIIA1 10.5.3/02

Author:	Schmuck, R.
Title:	Acute effects of a spray application of Bulldock EC025 on carabid beetles (*Poecilus cupreus*) under semifield conditions
Date:	13 August 1993
Doc ID:	M-052490-01-1
Report no.:	SXR/HF 88
Edition no.:	R-19130
Guidelines:	The experiment followed the protocol established by the German registration authority (BBA)
GLP:	yes
Validity:	not valid

Deviations: The study meets requirements from Heimbach *et al*., 2000 guideline, but application of reference treatment had no significant effect.
Test material: Beta-cyfluthrin EC 25 (Bulldock), as content: 27.8 g/L, batch no. 134 based on form. No. 04112/0004
Results: The results show that in an orchard situation, under normal management conditions, a spray application of Bulldock EC 25 applied at 450 mL/ha (12.5 g as/ha) poses no significant threat to carabid beetles, represented by *Poecilus cupreus*, active at ground level. This is mainly due to the low amount of spray deposits on to the ground as indicated by the failure of the reference substance to induce statistically significant effects.

Conclusion: As no effects in the reference treatment group were determined, the study is not valid according the current guideline Heimbach *et al*., 2000 (reference item should result in losses of at least 35 %).

KIIIA1 10.5.3/03

Author:	Schmuck, R.
Title:	Acute effects of a spray application of Bulldock EC025 on carabid beetles (*Poecilus cupreus*) under semifield conditions
Date:	1 April 1993
Doc ID:	M-052542-01-1
Report no.:	SXR/HF 91
Edition no.:	R-19129
Guidelines:	The experiment followed the protocol established by the former German registration authority (BBA)
GLP:	yes
Validity:	not valid

Deviations: The study meets requirements from Heimbach *et al*., 2000 guideline, but application of reference treatment had no significant effect

Test material: Beta-cyfluthrin EC 25 (Bulldock), as content: 27.8 g/L, batch no. 134 based on form. No. 04112/0004

Results: The results show that in half-grown winter rape fields, under normal management conditions, a spray application of Bulldock EC 25 applied at 300 mL/ha (8.3 g as/ha) poses no threat to carabid beetles, represented by *Poecilus cupreus*, active at ground level. This is mainly related to the low amount of spray fluid deposited on to the ground due to the dense vegetation cover during this crop stage as indicated by the failure of the reference substance to induce statistically significant effects.

Conclusion: As no effects in the reference treatment group were determined, the study is not valid according the current guideline Heimbach *et al*., 2000 (reference item should result in losses of at least 35 %).

KIIIA1 10.5.3/04

Author:	Schmuck, R.
Title:	Acute effects of a spray application of Bulldock EC025 on carabid beetles under semifield conditions
Date:	8 July 1992
Doc ID:	M-052680-01-1
Report no.:	SXR/HF 56
Edition no.:	R-19138
Guidelines:	No specific guideline
GLP:	yes
Validity:	additional information

Deviations: no guideline

Test material: Beta-cyfluthrin EC 25 (Bulldock), as content: 26.6 g/L, batch no. 134 based on form. No. 04112/0004

Results: The presented results demonstrate that a Bulldock EC 25 spray application against ear aphids in winter wheat (stadium with ear) does not affect the dominant carabid species (*Pterostichus madidus, Loricera pilicornis*) up to a rate of 300 mL/ha (8 g as/ha). In contrast, male and juvenile spiders may be impacted. This impact, however, is very short-lived. The data reveal that the web spiders' activity had completely returned to control level as soon as 2 weeks after application.

Conclusion: Additional information about effects on dominant in-field carbid beetles.

B.9.5.2.4 Field studies with non-target arthropods

KIIIA1 10.5.3/01

Author:	Redl, H.; Fuchs, A.
Title:	Auswirkungen einer Austriebsbehandlung von Reben auf den Raubmilbenbesatz Journal: Mitteilungen Klosterneuburg, Volume:42, Pages:228-230
Date:	1992
Doc ID:	M-090498-01-1
Report no.:	Lit. 6738
Edition no.:	R-19137
Guidelines:	No guideline
GLP:	no
Validity:	supplementary information

Results:

Test Formulation	Cyfluthrin 050 EC
Test object	Predatory mite fauna
Application rate	14 g as/ha
Exposure	Spray treatment of vineyard plots (15-50 plants) at sprouting
Result	No or only moderate impacts on predatory mite population at 4 weeks after treatment (53 - 124 % control value).

Conclusion:
Supplementary information:
The publication is only a study summary.
The formulation was applied in vineyard.
Thus, it is a different culture than in the proposed use pattern (cereals and potatoes).
Moreover, a different product than the representative formulation was used (with cyfluthrin instead of beta-cyfluthrin)

KIIIA1 10.5.3/02

Author:	Schmuck, R.
Title:	Evaluation of certain pesticides activity against the cotton whitefly, *bemesia tabaci* and associated natural enemies on cotton plants under field conditions in Assiut Journal: Assiut Journal of Agricultural Sciences, Volume:21, Issue:5, Pages:331-339

Date:	1990
Doc ID:	M-090543-01-1
Report no.:	Lit. 7098
Edition no.:	R-19134
Guidelines:	No specific guideline
GLP:	no
Validity:	supplementary information

Deviations: Not applicable

Results:

Test Formulation	Beta-cyfluthrin 012.5 EC
Test object	Leaf - dwelling natural enemies: e.g. Coccinella, Scymnus, Chrysoperla, Syrphus
Application rate	22.3 g as/ha
Exposure	Spray treatment of 42 m² plots in cotton fields (n=4)
Result	Reduction relative to the control: 2 DAT: 62 % 7 DAT: 26 % 14 DAT: 23 % 21 DAT: 21 %

Conclusion:
Supplementary information:
The publication is only a study summary.
The formulation was applied in cotton plant.
Thus, it is a different culture than in the proposed use pattern (cereals and potatoes).

KIIIA1 10.5.3/03 (newly submitted with the dossier)

Author:	Vinall, S.
Title:	A field trial to determine the effects of Baythroid EC 050 (50 g/L cyfluthrin) on the non-target arthropod fauna of a winter-sown cereal crop, following two applications during spring/summer
Date:	24 November 2005
Doc ID:	
Report no.:	IRV-04-1
Edition no.:	R-19598
Guidelines:	The study design was based on the current UK MAFF guidelines (Anon., 1991), with treatments applied to replicate plots of approximately 1 ha in size
GLP:	yes
Validity:	R3 (not reliable) according de Jong et al. 2010

Deviations: none

Dates of experimental work: 23 April 2004 to 18 June 2005

Executive Summary
Baythroid EC 050 is an emulsifiable concentrate formulation, nominally containing 50 g cyfluthrin/L. The aim of this study was to determine the effect of this insecticide on the natural non-target arthropod (NTA) communities present in a crop of winter cereals. Two treatment rates of Baythroid EC 050 were evaluated, each being a representative field rate of 500 mL/ha (25 g as/ha) and a drift rate, equivalent to 2.85 mL product/ha (0.1425 g as/ha). Each treatment rate was applied to a crop of winter

barley on two occasions, with a 16-day interval.

When Baythroid EC 050 was applied twice to a winter barley crop at 25 g cyfluthrin/ha, the product had adverse effects on populations of certain groups of non-target arthropod. Those most affected were rove beetles (Staphylinidae) and spiders (Araneae). For some species in these groups, population recovery was not seen within the season in 2004. Subsequently (in May 2005), recovery was demonstrated within a year of the initial application. When applied at a spray drift rate (0.1425 g cyfluthrin/ha), two applications of Baythroid EC 050 had no marked adverse effects on populations of any group of non-target arthropod.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Baythroid EC 050 (cyfluthrin)
Description:	Clear straw coloured liquid
Lot/Batch #:	040429/1
Purity:	53.63 g/L (measured)
2. Vehicle and/or positive control:	Control: untreated positive control: BASF Dimethoate 40: 400 g/L dimethoate

B: STUDY DESIGN AND METHODS

1. Trial site and test design

The trial took place in southern England during summer 2004 in a crop of winter barley and continued during spring 2005, in the subsequent crop, which was pea. There were four treatment variants; Baythroid EC 050, applied at either 500 mL/ha or 2.85 mL/ha, a toxic reference of BASF Dimethoate 40 (400 g/L dimethoate) applied at 850 mL/ha and a control that was left untreated. Sixteen plots of approximately one ha were marked out and each treatment assigned to four replicate plots in a randomised block design. Applications were made using a commercial hydraulic-boom sprayer in a volume of 200 L water/ha. Each treatment was applied to the crop on two occasions, with a 16-day interval (times T1 and T2, corresponding to 18 May and 3 June 2004, respectively). These applications coincided with the end of flowering (decimal growth stages 55-59) and the end of ear emergence (decimal growth stages 67-69) of the barley.

2. Sampling methods and sample processing

The non-target arthropods present in the crop were sampled using a range of techniques. These included pitfall traps (to provide activity-dependent data on the epigeal fauna over 2-day periods), a „Vortis‟ suction sampler (to provide density-dependent data on epigeal communities of Collembola), yellow sticky-traps (to provide activity-dependent data on flying insects over 2-day periods) and sweep nets (to provide density-dependent data on foliar-active arthropods). The invertebrates in the samples were subsequently identified to appropriate taxonomic levels in order to determine those groups whose abundance was significantly affected by the treatments. Samples were taken prior to T1 (in order to determine the level of homogeneity in the NTA populations) and then at intervals after both T1 and T2. During 2004, sampling continued up to 38 days after the treatment applications at T2 (DAT2), with additional pitfall trap sampling being carried out 53-55 DAT2, following harvest. Three additional pitfall-trap samples were taken during May 2005, with traps being set in the control and 500 mL product/ha Baythroid EC 050 plots. The final sample was taken within one year of the initial (T1) application.

3. Statistical calculations

For each sampling occasion, statistical analysis was performed on the data for those taxonomic groups that achieved a threshold of ≥ 10 individuals per replicate (mean) in the control. For the 2004 data, treatments were compared by one-way analysis of variance and where significant effects were seen ($\alpha = 0.05$), individual treatments were separated from the control using Dunnett‟s Test. For the 2005 data, there were only two treatments to be compared and analysis was by t-test for unmatched pairs.

For simplification, the following abbreviations have been used in tables:
DAT1 = Days after the first treatment application (i.e. after time T1)
DAT2 = Days after the second treatment application (i.e. after time T2)

II. RESULTS AND DISCUSSION

A. FINDINGS

Results:

Table B.9.5-25: Summary of data from pitfall trap samples in 2004

Baythroid EC 050 Field rate (500 mL/ha)			**Baythroid EC 050 Drift rate (2.85 mL/ha)**		**BASF Dimethoate 40 (850 mL/ha)**	
Sign. level	**Time at which no significant difference from control**		**Sign. level**	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**
Carabidae (Coleoptera)						
Agonum dorsale	ns	-	ns	-	**	> 2 DAT2
Amara plebja	ns	-		ns	-	ns
Bembidion lampros	**	2-4 DAT1	ns	-	**	> 2 DAT2
Nebria brevicollis	ns	-	ns	-	*	13-15 DAT2
Pterostichus melanarius	ns	-	ns	-	ns	ns
Total carabid adults	*	2-4 DAT1	ns	-	***	4-6 DAT2
Carabid larvae	ns	-	ns	-	**	13-15 DAT2
Staphylinidae (Coleoptera)						
Philonthus cognatus	**	7-9 DAT2 #	ns	-	***	> 23 DAT2
Philonthus laminatus	*	7-9 DAT2	ns	-	**	2-4 DAT2
Tachyporus hypnorum	**	0-2 DAT2 #	ns	-	***	> 15 DAT2
Tachyporus chrysomelinus	***	> 15 DAT2	*	7-9 DAT1	***	> 15 DAT2
Tachyporus larvae	***	> 23 DAT2	ns	-	***	> 23 DAT2
Tachinus rufipes	**	4-6 DAT2	ns	-	*	2-4 DAT1
Xantholininae	ns	-	ns	-	*	> 2 DAT2
Total staphylinid adults	***	2-4 DAT2 #	ns	-	***	21-23 DAT2
Total staphylinid larvae	**	> 55 DAT2	ns	-	*	53-55 DAT2

Linyphiidae (Araneae)						
Erigone spp.	***	> 55 DAT2 #	ns	-	***	36-38 DAT2
Lepthyphantes tenuis	ns	-	*	7-9 DAT2	**	13-15 DAT2
Milleriana inerrans	**	4-6 DAT2	ns	-	*	4-6 DAT2
Total linyphiid spiders	***	> 55 DAT2 #	ns	-	***	36-38 DAT2
Lycosidae (Araneae)						
Pardosa pullata	***	> 15 DAT2 #	ns	-	ns	-
Total lycosid spiders	***	> 15 DAT2 #	ns	-	*	2-4 DAT2
Tetragnathidae (Araneae)						
Pachygnatha degeeri	***	> 15 DAT2	ns	-	***	> 15 DAT2
Soil mites (Acari)	ns	-	ns	-	**	> 55 DAT2

Asterisks indicate where individual treatments differed significantly from the control. The number of asterisks represents the greatest significance level achieved during the post-treatment assessments (= P < 0.05; ** = P < 0.01; *** = P < 0.001) and „ns" indicates where treatments did not differ significantly from the control on any occasion. Where significant differences from the control were observed, the earliest sampling occasion during which no further significant differences were observed is indicated. The symbol > indicates that differences were still significant on the last date in 2004 that a taxon was present in sufficient numbers to warrant statistical analysis (not necessarily the last date of sampling).*
For the pitfall samples taken in the control and 500 mL/ha Baythroid EC 050 plots during May 2005, only numbers of Staphylinidae and Araneae were recorded. The symbol # indicates taxa that were trapped with a mean of ≥ 10 per plot in the control. There were no significant differences (P > 0.05) between control and treatment.

Table B.9.5-26: Summary of data from sticky-trap samples

	Baythroid EC 050 Field rate (500 mL/ha)		**Baythroid EC 050 Drift rate (2.85 mL/ha)**		**BASF Dimethoate 40 (850 mL/ha)**	
	Sign. level	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**
Parasitica (Hymenoptera)						
Aphidius type	ns	-	ns	-	*	36-38 DAT2
Ichneumonidae	ns	-	ns	-	ns	-
Other parasitica	ns	-	ns	-	ns	-
Total parasitica	ns	-	ns	-	ns	-
Empididae (Diptera)	ns	-	ns	-	*	13-15 DAT2
Heteroptera	ns	-	ns	-	ns	-
Linyphiidae (Araneae)	ns	-	ns	-	ns	-
Syrphidae (Diptera)	ns	-	ns	-	ns	-

See footnote to table on previous page.

Table B.9.5-27: Summary of data from suction sampling

	Baythroid EC 050 Field rate (500 mL/ha)		Baythroid EC 050 Drift rate (2.85 mL/ha)		BASF Dimethoate 40 (850 mL/ha)	
	Sign. level	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**
Collembola						
Entomobryoidea	ns	-	ns	-	*	> 33 DAT2
Symphypleona	ns	-	ns	-	**	21 DAT2
Total Collembola	ns	-	ns	-	*	> 33 DAT2

See footnote to table on previous page.

Table B.9.5-28: Summary of data from sweep-net sampling

	Baythroid EC 050 Field rate (500 mL/ha)		Baythroid EC 050 Drift rate (2.85 mL/ha)		BASF Dimethoate 40 (850 mL/ha)	
	Sign. level	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**	**Sign. level**	**Time at which no significant difference from control**
Parasitica (Hymenoptera)						
Total Parasitica	*	> 33 DAT2	ns	-	ns	-
Other groups						
Homoptera – aphids	***	> 33 DAT2	ns	-	***	> 33 DAT2
Diptera – adults	***	> 33 DAT2	*	12 DAT1	***	> 33 DAT2

See footnote to table on previous page.

Drift-rate Baythroid EC 050

The drift-rate treatment of Baythroid EC 050 had no marked effects on numbers of any groups of NTA in the crop and the numerical trends for catches in the drift-rate treatment and the untreated control remained similar throughout the trial.

Field-rate Baythroid EC 050

Ground beetles (Carabidae)

The field-rate had a minor effect on one species (Bembidion lampros). Numbers of this beetle were significantly reduced on only one occasion (first sampling after T1). Consequently, the total number of carabid adults was also statistically significantly lower than the control directly after T1, but not at any other time.

Rove beetles (Staphylinidae)

The five species of rove beetle (Staphylinidae) with the highest sampled numbers in the study (Tachyporus hypnorum, T. chrysomelinus, Tachinus rufipes, Philonthus cognatus, P. laminatus) were significantly affected by the field-rate. Hence, the total number of adult rove beetles was significantly affected (recovery was indicated with a peak in sampled numbers 9 DAT2).

Tachyporus hypnorum
Significantly reduced after T1 only. Following T2 the sampled numbers were similar to the control through to the end of the season. Numbers sampled in May 2005 (349 DAT2) did not differ significantly from the control.

Tachyporus chrysomelinus
Significantly reduced directly after T1, without recovery during the season. Since this is a spring-breeder, recovery during the season was unlikely. The samples in May 2005 (349 DAT2) showed similar numbers in the control and field-rate plots (although numbers in the control were below the statistical threshold). Numbers in May 2005 were similar to those sampled directly prior to T1 in May 2004. Being a migratory species, spreading out from field margins in early spring, such a population recovery was to be expected and appears to have occurred within a year of T1.

Philonthus cognatus
Significantly reduced directly after T1 and for a period after T2. A full recovery was not likely during the season as this is a spring-breeder. Samples in May 2005 (349 DAT2) showed no significant difference between control and field-rate. Numbers sampled in May 2005 were similar to those sampled directly pre-treatment in May 2004. Hence, recovery had occurred within a year of T1.

Philonthus laminatus and *Tachinus rufipes*
Significantly reduced directly after T1, without recovery during the season. Since these are spring-breeders, recovery within the season was unlikely. Samples taken in May 2005 (349 DAT2) from control and field-rate plots did not contain these species in suitable numbers for analysis. This may have been due to the change in crop use (cereals in 2004 followed by peas in 2005). Since related species such as *P. cognatus* and *T. chrysomelinus*, demonstrated an ability to recolonise the treated plots within a year of T1, it must be assumed that the same potential for recovery would also exist for other staphylinids such as *P. laminatus* and *T. rufipes*.

Spiders (Aranae)
Linyphiidae
Erigone spp. was severely depleted with no recovery within the season. Less abundant species such as *Milleriana inerrans* and *Lepthypantes tenuis* were less affected, numbers of the former only being significantly reduced on the first sampling after T1 and numbers of the latter not being significantly affected in any sampling. Total numbers of linyphiids (with *Erigone* spp. as the major constituent) were significantly reduced up to and including the last sampling in 2004. From pitfall samples taken in May 2005, full recovery of the linyphiid population (including *Erigone* spp.) was observed within a year of T1.

Lycosidae, Tetragnathidae
Some statistically significant reductions in sampled numbers of other spider groups (Lycosidae, Tetragnathidae) were also observed in 2004. Late-season numbers of these spiders in the control and field- rate plots were insufficient to allow judgement on recovery within the season. However, the samples taken in May 2005 demonstrated recovery within a year of T1.

Soil dwelling mesofauna, and aerial fauna
The field-rate did not have a significant effect on numbers of either soil mites (sampled in pitfall traps) or Collembola (sampled by suction sampler). The aerial fauna sampled above the crop using sticky traps yielded relatively low numbers around the times of the applications, but numbers increased steadily through June. None of the groups of wasp and fly were significantly affected.

Dimethoate toxic reference
The toxic reference had significant effects on ground beetles, rove beetles, spiders, soil mites and Collembola. The results demonstrate that the field contained a sensitive community of non-target arthropods, for which it was possible to show statistically significant effects. For ground and rove beetles, soil mites and Collembola, the effects were generally greater than the field-rate of the test item.

III. CONCLUSIONS

When Baythroid EC 050 was applied twice to a winter barley crop at 25 g cyfluthrin/ha, the product had adverse effects on populations of certain groups of non-target arthropod. Those most affected were rove beetles (Staphylinidae) and spiders (Araneae). For some species in these groups, population recovery was not seen within the season in 2004.
Subsequently (in May 2005), recovery was demonstrated within a year of the initial application. When applied at a spray drift rate (0.1425 g cyfluthrin/ha), two applications of Baythroid EC 050 had no marked adverse effects on populations of any group of non-target arthropod.

Reliablity:

The applied test formulation was not the representative product Bulldock EC 25, but the cyfluthrin formulation Baythroid EC 050. The interpretation of study results in terms of the representative use of Bulldock EC25 is associated with a high level of uncertainty.

Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis. In the following orders are lacking completely:

1. Lepidoptera
2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera
6. Dermaptera

Consequently, the study is classified as not reliable R3 (according de Jong, 2010).

KIIIA1 10.5.4/04 (newly submitted with the dossier)

Author:	Vinall, S.
Title:	A field trial to determine the effects of Baythroid EC 050 (50 g/L Cyfluthrin) on the non-target arthropod fauna of an orchard crop, following two applications during spring/summer
Date:	13 April 2006
Doc ID:	
Report no.:	IRV-04-1
Edition no.:	R-19592
Guidelines:	IOBC (Hassan, 1992), ESCORT (Barrett *et al*, 1994), Brown (1998), IOBC, EPPO and BART Joint Initiative (Candolfi *et al*, 2000)
GLP:	yes
Validity:	R3 (not reliable) according de Jong et al. 2010

Executive Summary

Test material was Baythroid EC 050 (containing 50 g cyfluthrin/L). The aim was to determine the effects of two applications, with a 14-day interval, on the natural, foliar-dwelling, non-target arthropod (NTA) communities present in a mature apple orchard. The intention was to evaluate both the initial effects on the key foliar-dwelling taxa within the orchard and the potential for within-season recovery.

conducted in a commercial orchard in south-west France. Arthropod populations were monitored throughout the growing season by both chemical inventory-sampling and leaf sampling.
Baythroid EC 050 was evaluated at four application rates. These were a representative field rate (25 g as/ha), drift rate of 3.9325 g as/ha, drift rate of 0.9 g as/ha and drift rate of 0.2725 g as/ha. These treatments were compared to a water-treated control, and a toxic reference (dimethoate). All treatments were applied twice, with a 14-day interval between applications.

Field rate treatment of Baythroid EC 050 (2 applications at 25 g as/ha)
For the field-rate application of Baythroid EC 050, effects were observed for a range on non-target arthropods. Effects were mostly observed shortly after treatment, due to acute mode of action of the test material. For highly mobile groups (adult Diptera, Hymenoptera and Coleoptera) rapid recovery occurred. This indicates a short persistence of foliar residues of the test material. For less mobile organisms (with relatively long development times) like larval Coccinellidae, recovery occurred within the season.

High drift rate of Baythroid EC 050 (2 applications at 3.9325 g as/ha)
Overall, treatment-related effects were observed for a limited number of taxonomic groups (much less than for the field-rate). Recovery (or potential for recovery) has been observed within the season.

Middle drift rate of Baythroid EC 050 (2 applications at 0.9 g as/ha)
Overall, the influence of middle drift-rate treatment on the non-target arthropod community was negligible.

Lowest drift rate of Baythroid EC 050 (2 applications at 0.2725 g as/ha)
Overall, it is considered that the lowest drift-rate had no effect on the non-target arthropod community.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	Baythroid EC 050 (cyfluthrin)
Description:	Clear straw coloured liquid
Lot/Batch #:	60111156
Purity:	49.8 g/L (measured)
2. Vehicle and/or positive control:	Control: untreated (tap water) Positive control: Technoate 40EC (400 g/L dimethoate)

B: STUDY DESIGN AND METHODS

1. Trial site and test design
The trial was carried out in a commercial apple orchard in Monheurt in the Garonne Valley, south-west France. The orchard (approx. 5.75 ha) contained mature trees approximately 2.0-2.5 m in height. The main crop area was divided into 22 plots. Twenty of these were around 0.24 ha in size, each containing approximately 485 trees. These plots, arranged as four blocks of five plots, were assigned to the control and the four test item treatments in a randomised block design. Two additional plots of trees alongside the main blocks (measuring approximately 0.41 ha and 0.13 ha, respectively) were assigned to a toxic reference treatment.
Five principal treatments were evaluated, each being applied to four replicate plots:
- a control (water)

- Baythroid EC 050 applied at a rate of 25 g as/ha (500 mL product/ha).
- Baythroid EC 050 applied at a rate of 3.9325 g as/ha (78.65 mL product/ha).

- Baythroid EC 050 applied at a rate of 0.9 g as/ha (18 mL product/ha).
- Baythroid EC 050 applied at a rate of 0.2725 g as/ha (5.45 mL product/ha).

A toxic reference item (dimethoate) was applied to two replicate plots adjacent to the main trial area. Each treatment was applied at 1000 L spray solution/ha.
The spray dates were 16 May 2005 and 30 May 2005. The BBCH crop growth stages at the time of each application were 72 and 73-74, respectively (Bleiholder, 1997).
Applications were made using a tractor-mounted broadcast air-assisted sprayer. A full calibration of the output from the moving sprayer was made in advance of treatments. Environmental conditions were recorded throughout the trial using an electronic weather station.

2. Sampling methods and sample processing
Inventory sampling: This was to quantify invertebrate populations on the sprayed trees, both before treatment and for the rest of the season. On each sampling occasion, pairs of collecting-sheets were pegged out under three randomly-selected trees in each plot. With one sheet placed each side of the trunk, any invertebrates falling down were collected. In the evening, an application of dichlorvos was made to these trees using a motorised sprayer fitted with a hand lance. The day after the dichlorvos application, all of the arthropods on the sheets were collected by washing them into a bucket and then straining the contents through a sieve. The arthropods were transferred to jars containing 70 % ethanol. No further sampling was made from the same trees. Sampling was performed only on trees located in the central part of each plot. The inventory samples (10 in total) were taken 7 days before the first application, 3 days before the second application and 3, 11, 24, 46, 65, 85, 122 and 149/150 days after the second application. Samples in the toxic reference treatment were only taken on the first five dates.

Leaf sampling: This was to quantify populations of mites. On each sampling occasion, 240 leaves per replicate plot were collected, being taken from a minimum of four trees per plot. The leaves were put into jars and transported to the field laboratory. To extract mites, a washing technique was used or Berlese-Tullgren funnel apparatus, before being transferred to a preservative. The leaf samples (10 in total) were taken 6 days before the first application, 3 days before the second application and 3, 11, 24, 45, 60, 85, 121 and 150 days after the second application. Samples in the toxic reference treatment were only taken on the first five dates.

3. Statistical calculations
Taxonomic identification of the arthropod fauna present in the samples was then carried out. The numbers of individual taxa in the test item treatments were compared to those in the control on a date-by-date basis, by one-way analysis of variance (ANOVA) of the log-transformed data. Dunnett‟s test was used to confirm where treatments differed significantly from the control ($\alpha = 0.05$). For the toxic reference treatment (which was not part of the randomised block test design), comparisons with the control treatment were made on a date-by-date basis using t-test for unmatched pairs.

II. RESULTS AND DISCUSSION

A. FINDINGS
Results are summarised in the following Tables. For the test item treatments, this includes a comment on whether the statistically significant differences from the control were considered to be treatment-related. The timing of effects and recovery of each taxonomic group is summarised in the Conclusions.
Results:

Table B.9.5-29: Summary of data from inventory samples – part 1

	Baythroid EC 050 (cyfluthrin)								**dimethoate**
	Field rate (25 g as/ha)		**High drift rate (3.93 g as/ha)**		**Middle drift rate (0.9 g as/ha)**		**Low drift rate (0.273 g as/ha)**		**(400 g as/ha)**
	Sig. level	***Treat-ment related ?***	***Sig. level***	***Treat-ment related ?***	***Sig. level***	***Treat-ment related ?***	***Sig. level***	***Treat-ment related?***	***Sig. level***
Diptera									
Total adult Diptera	*	yes	ns		ns		ns		ns
Cecidomyiidae	ns		ns		ns		ns		ns
Chironomidae	ns		ns		ns		ns		*
Chloropidae	ns		ns		ns		ns		ns
Drosophilidae	***	yes	ns		ns		ns		ns
Empididae	*	yes	ns		ns		ns		ns
Lauxaniidae	***	yes	***	yes	**	yes	ns		*
Muscidae/Anthomyidae	ns		ns		ns		ns		ns
Mycetophilidae	**	yes	ns		ns		ns		*
Phoridae	ns		ns		ns		ns		*
Psychodidae	ns		ns		ns		ns		ns
Tipulidae	ns		ns		ns		ns		-
Syrphidae (adults)	ns		ns		ns		ns		ns
Larval Diptera: Syrphidae	ns		ns		ns		ns		**
other flies	**	yes	*	yes	ns		ns		*
Hymenoptera									
Total Hymenoptera	ns		ns		ns		ns		ns
Ichneumonid.: Ichneumonidae	ns		ns		ns		ns		ns
Braconidae	*	yes	ns		ns		ns		ns
Hemiptera									
Total Hemiptera excl. aphids (but incl. phytophagous species)	**	yes	*	yes	ns		ns		ns
Cixiidae (phytophagous)	*	yes	ns		ns		ns		ns
Cicadellidae (phytophagous)	**	yes	ns		ns		ns		**
Cimicidae	*	yes	**	yes	ns		ns		ns
Lygaeidae (phytophagous)	ns		ns		ns		ns		-
Pentatomidae (phytophagous)	ns		ns		ns		ns		ns
Aphidae (aphids)	ns		ns		ns		ns		ns

Table B.9.5-30: Summary of data from inventory samples – part 2

	Baythroid EC 050 (cyfluthrin)								**dimethoate**
	Field rate (25 g as/ha)		**High drift rate (3.93 g as/ha)**		**Middle drift rate (0.9 g as/ha)**		**Low drift rate (0.273 g as/ha)**		**(400 g as/ha)**
	Sig. level	***Treat-ment related ?***	***Sig. level***	***Tre at-me nt rela ted?***	***Sig. level***	***Treat-ment related ?***	***Sig. level***	***Treat-ment related?***	***Sig. level***
Psocoptera	ns		ns		ns		ns		-
Coleoptera									
Total Coleoptera adults	**	yes	ns		ns		ns		**
Bruchidae	ns		ns		ns		ns		-
Carabidae	ns		ns		ns		ns		ns
Chrysomelidae	ns		ns		ns		ns		-
Coccinellidae: *C. 7-punctata*	ns		ns		ns		ns		-
other adults	ns		ns		ns		ns		ns
total larvae	***	yes	*	yes	ns		ns		ns
Curculionidae	ns		ns		ns		ns		**
Lathrididae	***	yes	ns		ns		ns		**
Staphylinidae: *Tachyporus*	***	yes	ns		ns		***	no	**
other adults	ns		ns		ns		ns		ns
Coleoptera larvae (exc. Cocc.)	ns		ns		ns		ns		ns
Lepidoptera (adults)	ns		ns		ns		ns		-
Neuroptera									
Hemerobiidae	ns		ns		ns		ns		ns
lacewing larvae	***	yes	*		yes		ns	ns	**
Thysanoptera (phytophagous)	**	yes	ns		ns		ns		*
Araneae									
Total spiders	*	yes	ns		ns		ns		ns
Araneidae	*	yes	ns		ns		ns		ns
Dictynidae	ns		ns		ns		ns		-
Oxyopidae	ns		ns		ns		ns		-
Salticidae	ns		ns		ns		ns		*
Thomisidae	ns		ns		ns		ns		ns
Acari									
Trombididae	ns		ns		ns		ns		ns

Asterisks indicate where individual treatments differed significantly from the control on any one date. The number of asterisks represents the greatest significance level achieved during the post-treatment assessments (= P < 0.05; ** = P < 0.01; *** = P < 0.001) and „ns" indicates where treatments did not differ significantly from the control on any occasion. For the toxic reference treatment, – indicates threshold for analysis not met on dates when samples were made. „Yes/no" indicates whether the significant effect was considered to be treatment related.*

Table B.9.5-31: Summary of data from leaf samples

	Baythroid EC 050 (cyfluthrin)								**dimethoate**
	Field rate (25 g as/ha)		**High drift rate (3.93 g as/ha)**		**Middle drift rate (0.9 g as/ha)**		**Low drift rate (0.273 g as/ha)**		**(400 g as/ha)**
	Sig. level	***Treat-ment related ?***	***Sig. level***	***Treat-ment related?***	***Sig. level***	***Treat-ment related ?***	***Sig. level***	***Treat-ment related?***	***Sig. level***
Acari									
Mesostigmata : Phytoseiidae									
Amblyseius andersoni	ns		*	no	ns		ns		ns
Prostigmata : Tetranychidae	ns		ns		ns		ns		-
Prostigmata : Eriophyidae	*	no	ns		ns		ns		ns
Prostigmata : Tydeidae	ns		ns		ns		ns		-

Asterisks indicate where individual treatments differed significantly from the control on any one date. The number of asterisks represents the greatest significance level achieved during the post-treatment assessments (= P < 0.05; ** = P < 0.01; *** = P < 0.001) and „ns" indicates where treatments did not differ significantly from the control on any occasion. For the toxic reference treatment, – indicates threshold for analysis not met on dates when samples were made. „Yes/no" indicates whether the significant effect was considered to be treatment related.*

III. CONCLUSIONS

The effect of applying Baythroid EC 050 twice, in May, to an apple orchard at a typical field rate of 500 mL/ha (25 g cyfluthrin/ha) and three drift rates (78.65, 18 and 5.45 mL product/ha) is summarised below.

Field rate treatment of Baythroid EC 050 (2 applications at 25 g as/ha)
For the field-rate application of Baythroid EC 050, effects were observed for a range on non-target arthropods. Effects were mostly observed shortly after treatment, due to acute mode of action of the test material. For highly mobile groups (adult Diptera, Hymenoptera and Coleoptera) rapid recovery occurred. This indicates a short persistence of foliar residues of the test material. For less mobile organisms (with relatively long development times) like larval Coccinellidae, recovery occurred within the season.

Taxonomic group	**Effects**	**Recovery**
Total of 58 groups statistically analysed (54 in inventory samples; 4 in leaf samples)	19 groups gave at least one statistically significant reduction.	See below
	15 were considered to be treatment-related effects on non-target arthropods (see below).	
Total adult Diptera	+3 DAT1 (i.e. -11 DAT2)	+3 DAT2
Drosophilidae (Diptera)	+3 DAT1 (i.e. -11 DAT2)	+3 DAT2
Empididae (Diptera)	+3 DAT2	+11 DAT2
Lauxaniidae (Diptera)	+3, +11, +24 DAT2	Signs of recovery at +24 DAT2, before natural decline. (Lack of effects in other adult Diptera should be noted).
Mycetophilidae (Diptera)	+3 DAT1 (i.e. -11 DAT2)	+3 DAT2

Larval Diptera (excluding Syrphidae)	+11, +46 DAT2	+24 DAT2 (Signs of recovery before natural decline due to development into adults). +149/150 DAT2 (Recovery confirmed by appearance of second generation within season).
Braconidae (Hymenoptera)	+3 DAT1 (i.e. -11 DAT2)	+3 DAT2
Cimicidae (Hemiptera*)	+24 DAT2	+46 DAT2. However, identification of recovery complicated by natural decline in population. Potential for recovery of Cimicidae confirmed by recovery of other Hemiptera.
Total adult Coleoptera	+3 DAT1 (i.e. -11 DAT2)	+3 DAT2
Larval Coccinellidae (Coleoptera)	+3 DAT2	+85 DAT2 (Following the effect, there was a natural decline due to development into adults. Second generation showed recovery at +85 DAT2).
Lathridiidae (Coleoptera)	+3 DAT1 (i.e. -11 DAT2), +11 DAT2	+24 DAT2
Tachyporus spp. (Coleoptera)	+24 DAT2	+149/150 DAT2 (Very low numbers in control for all except two sampling dates, and variation of results between lower treatment rates, raises questions on the biological significance of the „effect").
Larval Neuroptera	+3 DAT2, +11DAT2	+24 DAT2
Total Araneae	+46 DAT2	+85 DAT2
Araneidae (Araneae)	+46 DAT2	+65/+85 DAT2

Effects: Sample dates when statistically significant effects were seen, when compared to the control treatment.
Recovery: The earliest sampling occasion when no further statistically significant differences were observed.
DAT1 = days after treatment 1, DAT2 = days after treatment 2.
* „Total Hemiptera (excluding aphids)" and three families within this larger grouping gave significant differences from the control. However, the Hemiptera identified were predominantly phytophagous (plant-feeding) species and were not therefore considered as being *non-target arthropods.* The Cimicidae was the only hemipteran family containing predominantly predatory species and therefore considered as *non-target arthropods*.

Highest drift rate of Baythroid EC 050 (2 applications at 3.9325 g as/ha)

Overall, treatment-related effects were observed for a limited number of taxonomic groups (much less than for the field-rate). Recovery (or potential for recovery) has been observed within the season.

Taxonomic group	**Effects**	**Recovery**
Total of 58 groups statistically analysed (54 in inventory samples; 4 in leaf samples)	8 groups gave at least one statistically significant reduction. 5 were considered to be treatment-related effects on non-target arthropods (see below).	See below
Lauxaniidae (Diptera)	+3, +11, +24 DAT2	Signs of recovery at +24 DAT2, before natural decline. (Lack of effects in other adult Diptera should be noted).
Larval Diptera (excluding Syrphidae)	+11 DAT2	+24 DAT2
Cimicidae (Hemiptera*)	+24 DAT2	+46 DAT2. However, identification of recovery complicated by natural decline in population. Potential for recovery of Cimicidae confirmed by recovery of other Hemiptera.

Larval Coccinellidae (Coleoptera)	+11, +24 DAT2	+85 DAT2 (Following the effect, there was a natural decline due to development into adults. Second generation showed recovery at +85 DAT2).
Larval Neuroptera	+11 DAT2	+24 DAT2

Effects: Sample dates when statistically significant effects were seen, when compared to the control treatment.
Recovery: The earliest sampling occasion when no further statistically significant differences were observed.
DAT2 = days after treatment 2.
* „Total Hemiptera (excluding aphids)" gave significant differences from the control. However, the Hemiptera identified were predominantly phytophagous (plant-feeding) species and were not therefore considered as being *non-target arthropods*. The Cimicidae was the only hemipteran family containing predominantly predatory species and therefore considered as *non-target arthropods*.

Middle drift rate of Baythroid EC 050 (2 applications at 0.9 g as/ha)
Overall, the influence of middle drift-rate treatment on the non-target arthropod community was negligible.

Taxonomic group	**Effects**	**Recovery**
Total of 58 groups statistically analysed (54 in inventory samples; 4 in leaf samples)	1 group gave a statistically significant reduction (see below).	See below
Lauxaniidae (Diptera)	+3 DAT2	+11 DAT2

Lowest drift rate of Baythroid EC 050 (2 applications at 0.2725 g as/ha)
Overall, it is considered that the lowest drift-rate had no effect on the non-target arthropod community.

Taxonomic group	**Effects**	**Recovery**
Total of 58 groups statistically analysed (54 in inventory samples; 4 in leaf samples)	1 group gave one statistically significant reduction (*Tachyporus* spp.), but it was not considered to be treatment-related.	Not applicable

Effects: Sample dates when statistically significant effects were seen, when compared to the control treatment.
Recovery: The earliest sampling occasion when no further statistically significant differences were observed.

Reliability:

However, the study was conducted in orchard. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

The applied test formulation was not the representative product Bulldock EC 25, but the cyfluthrin formulation Baythroid EC 050. The interpretation of study results in terms of the representative use of Bulldock EC25 is associated with a high level of uncertainty.

Arthropods caught are only out of 8 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis
The following orders are lacking completely:

1. Orthoptera
2. Thysanoptera
3. Dermaptera
4. Collembola

Consequently, the study is classified as not reliable R3 (according de Jong, 2010).

KIIIA1 10.5.3/05 (newly submitted with the dossier)

Author:	Mack P.
Title:	A field study assessing the impact of Bulldock 25 EC on the non-target arthropod fauna in an alfalfa field in Spain
Date:	13 April 2006
Doc ID:	
Report no.:	S12-01037
Edition no.:	R-28693
Guidelines:	Candolfi *et al.* (2000), de Jong *et al.* (2010)
GLP:	yes
Validity:	R3 (not reliable) according de Jong et al. 2010

Executive Summary

Bulldock 25 EC is an emulsifiable concentrate formulation, nominally containing 25 g beta-cyfluthrin/L. The aim of this study was to determine the effect of this insecticide on the natural non-target arthropod (NTA) communities present in an alfalfa field. Bulldock 25 EC was applied two times with a 15-day interval, each time with a field rate of 0.5 L product/ha (12.5 g as/ha, nominal).
Bulldock 25 EC applied twice to an alfalfa field at nominally 0.5 L product/ha (12.5 g as/ha) resulted in slight and transient effects (class 2; de Jong et al., 2010) and pronounced short term effects (class 3; de Jong et al., 2010). The multivariate analysis of the results revealed no statistically significant effect of the test item treatment on the community of non-target arthropods.
For Pitfall traps and Vortis suction sampling the majority of effects occurred 14 and 21 days after the last application. No effects of the test item treatment were observed at the last two samplings (112 and 140 DALA) for any of the sampling methods.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC
Description:	Liquid / yellow
Lot/Batch #:	92110164
Purity:	25 g/L (nominal), 24.79 g/L (measured)

2. Vehicle and/or positive control: Control: water sprayed Reference item: Perfektion: 37.4 % (w/w) dimethoate

B: STUDY DESIGN AND METHODS

1. Trial site and test design
The trial took place near Almansa, Spain, from mid of May until the end of October in alfalfa. The trial included one test item group with Bulldock 25 EC (T), applied twice at 0.5 L product/ha (12.5 g as/ha), a water treated control and a reference item treatment (Perfekthion, as dimethoate), applied twice at 4 L product/ha. Every treatment group consisted of 4 replicates. Plot sizes were approximately 10000 m_2. Applications were made using a calibrated boom sprayer (Agricur AB 30) with a spray

volume of 200 L water/ha. Each treatment was applied to the crop on two occasions, with a 15-day interval (23 May and 07 June 2012).

2. Sampling methods and sample processing
The non-target arthropods present in the crop were sampled using three different techniques. These included pitfall traps (to provide data on soil surface active arthropods), visual control assessments (beating) (to provide data on arthropods living on the crop) and Vortis suction sampling (to provide data on foliage and ground-dwelling arthropods). For each sampling technique the 1st sampling was conducted prior to the 1st application. Pitfall trap sampling took place 8 times over the course of the trial. The traps were active for a 6 to 8-day interval for each sampling. Visual control assessments (beating) were taken at approximately weekly intervals for the first samplings and at two week intervals towards the end of the study, resulting in 12 visual control assessments over the course of the study. For the Vortis suction sampling eight samplings were performed over the course of the study. The invertebrates in the samples from the pitfall traps and the inventory sampling were transferred into 70 % ethanol and subsequently identified to appropriate taxonomic levels.

3. Statistical calculations
Statistical analyses of abundances on individual taxon level, higher taxonomic groups and total abundance for each sampling occasion were performed using univariate analysis. Comparisons were undertaken between test item treatment versus control and reference item versus control. Additionally, multivariate statistical methods were used to analyse effects on community level: Principal Component Analysis (PCA) and Principal Response Curves (PRC).

II. RESULTS AND DISCUSSION

A. FINDINGS

Reference item
The applied reference item treatment gave a reduction for a number of arthropod taxa, especially in the first samplings. In the Pitfall traps 19 of 26 taxa analysed were statistically significantly reduced, including the total number of arthropods caught. Statistically significantly higher abundances were observed for one taxon at the last sampling (140 DALA). In the visual control (beating) 7 of the 10 analysed taxa were statistically significantly reduced, most of them in the first assessments. In the Vortis suction sampling abundances of 11 taxa out of 21 (including the total catch) were statistically significantly reduced by the reference item treatment. One taxon showed a statistically significant increase in abundances without reductions in previous samplings. Additionally for all sampling methods there were more than 10 % of the taxa analysed with a reduction of more than 50 % at least once. The PRC for the Pitfall trap sampling data was not statistically significant. For the Vortis suction sampling data the PRC confirmed a statistically significant influence of the reference item treatment on the arthropod community at the 2nd, 3rd and 4th sampling (4, 14 and 21 DALA). Further the combined PRC (Pitfall traps and Vortis suction sampling), showed a statistically significant effect of the reference item treatment at the 2nd, 3rd, 4th and 7th sampling (8, 14, 21 and 84 DALA). Therefore, the reference item proved that the test system was sensitive to the application of an insecticide.

Multivariate analysis (PRC)
According to the multivariate analysis (PRC) the test item treatment had no impact on the ground and plant living arthropod community evaluated with two of the three sampling methods (Visual control assessments were not evaluated by multivariate statistics, as the number of taxa was not sufficient for analyses).

Summary of pitfall trap sampling
The abundance of all arthropods caught in pitfall traps was not affected to a statistically significant extent by the applications of the test item treatment.

Table B.9.5-32: Most abundant arthropods (n ≥ 192) caught with pitfall traps and statistically significant differences to the control

Taxon		Sampling days after last application							
		16 DBLA	8 DALA	14 DALA	21 DALA	42 DALA	56 DALA	84 DALA	140 DALA
Total catch			R	R	R	R			
Araneae total			T	R	R	R			
Gnaphosidae total			R	T, R	R			R	
Setaphis carmeli	Adult								
Lycosidae total				R					
Myriapoda	Adult								
Coleoptera total			R						
Anthicidae	Adult								
Carabidae total									
Calathus ambiguus	Adult								
Harpalus affinis	Adult		R#	R	R	T, R			
Coccinellidae total			R	R#	T+, R				
Coccinellidae*	Adult								
Coccinellidae*	Juvenile		R	R#	T+, R#				
Curculionidae*(p)	Adult		T+	T+					R#
Silphidae	Adult		R						
Staphylinidae total			R#		R				
*Xantholinus semirufus**	Adult				R#				
Diptera total			R						
Brachycera	Adult		R						R+
Hymenoptera total			R	R	R	R			
Hymenoptera	Adult		R						
Formicidae	Adult		R	R	R	R			
Auchenorrhyncha Cicadellidae (p)	Adult		T	R	T, R		R		
Sternorrhyncha Aphididae (p)	Adult			R	R				
Isopoda	Adult								

DBLA = days before last application; DALA = days after last application
T: statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment (2 x 12.5 g as/ha)
R: statistically significant decrease compared to the control ($p \leq 0.05$) of Reference item
+ behind T or R means statistically significant increase compared to the control (t-test, $p \leq 0.05$)
# behind T or R means statistically significant difference for the Wilcoxon-test ($p \leq 0.05$)
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Applications were performed on 23 May 2012 and 07 June 2012
* added taxa due to Canoco analysis, but not matching the criteria n ≥ 192
(p) Pest species / Some species are considered as serious pest

Four taxa (Aranae, Gnaphosidae, *Harpalus affinis* and Cicadellidae) showed a statistical significant reduction between test item treatment and control. With the exception of the cicadas this significant reduction was observed at a single sampling date. The family Cicadellidae (Auchenorrhyncha) was decreased to a statistically significant extent at the 2nd and 4th sampling (8 and 21 DALA) in the test item treatment compared to the control. No further statistically significant differences to the control were observed, although abundances were below control level until the 7th sampling (84 DALA). This effect is most likely treatment related; as cicadas are affected by the test item through their way of nutrition (they suck sap from the xylem). Three taxa were present in statistically significantly higher

abundances at least at a single sampling date: the total Coccinellidae (ladybirds), juvenile Coccinellidae and adult Curculionidae. Afterwards abundances were comparable to the control again, without further statistically significant differences. All other taxa were not affected.

Summary of visual control assessment (beating)

Of the 10 taxa analysed six showed a statistically significant difference between the test item treatment and the control. All other taxa were not affected.

Table B.9.5-33: Most abundant arthropods (n ≥ 89) caught with Visual control (Beating) and statistically significant difference to the control

Taxon	Sampling days after last application					
	16 DBLA	**8 DBLA**	**4 DALA**	**8 DALA**	**14 DALA**	**21 DALA**
Araneae total				T, R		
Coleoptera total				R		
Coleoptera (undet.) (ad.)				R		
Heteroptera total		R		T, R	T	
Heteroptera (others)				T, R		
Anthocoridae (ad.)		R			T	R
Lepidoptera total				T#	T	
Lepidoptera (larv.)				T#	T	
Sternorrhyncha **Aphididae total (p)**			R	R#	R	
Aphididae (para.) (p)						
Araneae total						
Coleoptera total				T		
Coleoptera (undet.) (ad.)						
Heteroptera total						
Heteroptera (others)						
Anthocoridae (ad.)	R					
Lepidoptera total	T					
Lepidoptera (larv.)	T					
Sternorrhyncha Aphididae total (p)						
Aphididae (para.) (p)						

DBLA = days before last application; DALA = days after last application
T: statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment (2 x 12.5 g as/ha);
R: statistically significant decrease compared to the control ($p \leq 0.05$) of Reference item
behind T or R means statistically significant difference for the Wilcoxon-test ($p \leq 0.05$)
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Applications were performed on 23 May 2012 and 07 June 2012
(p) Pest species / Some species are considered as serious pest

Araneae, Coleoptera, Anthocoridae and undetermined Heteroptera showed a significant reduction at a single sampling date. For the order Heteroptera statistically significantly lower numbers were observed in the test item treatment compared to the control at the 4th and 5th assessment (8 and 14 DALA). This difference could be treatment related. However, abundances were comparable to the control for all subsequent assessments. The order Lepidoptera and the juvenile Lepidoptera were present at statistically significantly lower abundances in the test item treatment compared to the control at the 4th, 5th and 7th assessment (8, 14 and 29 DALA). This difference might be treatment related, as abundances were comparable to the control at the 1st sampling (16 DBLA) and declined after the first application. A recovery was visible in later samplings, but abundances remained below

control level.

Summary of Vortis suction sampling
The abundance of all arthropods caught with Vortis suction sampling showed no statistically significant effect of the test item treatment when compared to the control.

Table B.9.5-34: Most abundant arthropods (n ≥ 128) caught with Vortis suction sampling and their significant differences to the control

Taxon	Sampling days after last application							
	16 DBLA	8 DALA	14 DALA	21 DALA	42 DALA	56 DALA	84 DALA	144 DALA
Total catch		R	R	R				
Araneae total						T		
Linyphiidae total**								R
Linyphiidae (juv)**								R
Coleoptera total				T+#				
Coccinellidae total			R	T+#, R#				
Coccinellidae (juv)*				T+#				
*Coccinella septempunctata**								
*Hippodamia variegata**								
Corylophidae								
Curculionidae (p)		R+			R+			
Diptera total	T	T, R						
Hymenoptera total								
Encyrtidae*		R#	R					
Eulophidae*	T#		R					
Formicidae						R		R
Scelionidae*			R			R+		
Auchenorrhyncha total			T					
Cicadellidae (p)			T					
Aphididae (p)		R	R					
Collembola				R			R	

DBLA = days before last application; DALA = days after last application
T: statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment (2 x 12.5 g as/ha);
R: statistically significant decrease compared to the control ($p \leq 0.05$) of reference item
+ behind T or R means statistically significant increase compared to the control (t-test, $p \leq 0.05$)
# behind T or R means statistically significant difference for the Wilcoxon-test ($p \leq 0.05$)
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Applications were performed on 23 May 2012 and 07 June 2012
* added taxa due to Canoco analysis, but not matching the criteria n ≥ 128
** added to analysis, but not matching the criteria n ≥ 128
(p) Pest species / Some species are considered as serious pest

Of the 21 taxa analysed five (Araneae, Diptera, Hymenoptera, Auchenorrhyncha and Cicadellidae) showed a statistically significant reduction between the test item treatment and the control. Over the whole course of the study these taxa showed low abundances, with a significant reduction at a single sampling date. No further statistically significant differences to the control were found in later samplings. Statistically significantly higher numbers were found for three taxa (Coleoptera, Coccinellidae and juvenile Coccinellidae). This was most likely due to chance or population fluctuations. No further statistically significant differences were observed. All other taxa analysed were not affected.

III. CONCLUSIONS

Bulldock 25 EC applied twice to an alfalfa field at nominally 0.5 L product/ha (12.5 g as/ha) resulted in slight and transient effects (class 2; de Jong et al., 2010) and pronounced short term effects (class 3; de Jong et al., 2010). The multivariate analysis of the results revealed no statistically significant effect of the test item treatment on the community of non-target arthropods.
For Pitfall traps and Vortis suction sampling the majority of effects occurred 14 and 21 days after the last application. No effects of the test item treatment were observed at the last two samplings (112 and 140 DALA) for any of the sampling methods.

Reliablity:

However, the study was conducted in alfalfa. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

Arthropods caught are only out of 7 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis.

The following orders are lacking completely:
1. Dermaptera
2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera

The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

Consequently, the study is classified as not reliable R3 (according de Jong, 2010).

KIIIA1 10.5.3/06 (newly submitted with the dossier)

Author:	Knäbe, S.
Title:	A field study assessing the impact of Bulldock 25 EC on the non-target arthropod fauna in pome fruit orchard in Germany
Date:	12 September 2013
Doc ID:	
Report no.:	S12-01040
Edition no.:	R-28694
Guidelines:	Candolfi *et al.* (2000), de Jong *et al.* (2010)
GLP:	yes
Validity:	R3 (not reliable) according de Jong et al. 2010

Dates of experimental work: 18 May 2012 to 29 November 2012

When Bulldock 25 EC was applied twice to a pome fruit orchard at 17.5 g beta-cyfluthrin/ha, treatment related effects were observed on certain taxa of non-target arthropod. Those were

Aphididae, Opiliones and Cicadellidae. The duration of the sampling in 2012 (approximately 5 months) was sufficient to demonstrate recovery for the community (excluding the family Cicadellidae). Effects on Cicadellidae were considered acceptable since cicadas are a family targeted by the test item.
Therefore, it can be concluded that non-target arthropods were affected by pronounced effects with a recovery within 4 months after the last application (class 4; de Jong et al., 2010).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC
Description:	Liquid / yellow
Lot/Batch #:	92110164
Purity:	25 g/L (nominal), 24.79 g/L (measured)
2. Vehicle and/or positive control:	Control: water sprayed Reference item 1: Calypso: 480 g/L thiacloprid Reference item 2: Spruzit Neu: 4.59 g/L pyrethrine

B. STUDY DESIGN AND METHODS

1. Trial site and test design
The field study was carried out in a pome fruit orchard in Hechthausen, Northern Germany from end of May until end of October 2012. The trial included one test item group with Bulldock 25 EC (T), a water treated control and a reference item treatment (Calypso, as thiacloprid, for the first application and Spruzit Neu, as pyrethrine, for the second application). The test item treatment and the water treated control comprised four plots (replicates). The reference item treatment comprised three plots (replicates). Plot sizes were 2299.5 m2. Applications were made using a calibrated air blast sprayer (Lochmann) with a volume of 500 L water/ha. Each treatment was applied to the crop on two occasions, with a 14-day interval (07 June and 21 June 2012).

2. Sampling methods and sample processing
The non-target arthropods present in the crop were sampled using three different techniques. These included pitfall traps (to provide data on soil surface active arthropods), visual assessments examining methodically upper and lower surfaces of leaves (to provide data on foliage-dwelling arthropods, e.g. predatory/parasitic arthropods) and inventory sampling (to provide data of the whole arthropod community within the trees). For each sampling technique the 1st sampling was conducted prior to the 1st application. Pitfall trap sampling took place 6 times over the course of the trial. The traps were active for a 7-day interval for each sampling. Visual assessments were taken at approximately two week intervals for the first two samplings, weekly intervals for subsequent samplings and again at a two week interval towards the end of the sampling period, resulting in 16 visual assessments over the course of the study. For the inventory sampling six samplings were performed over the course of the study. The invertebrates in the samples from the pitfall traps and the inventory sampling were subsequently identified to appropriate taxonomic levels.

3. Statistical calculations:
Statistical analyses of abundances on individual taxon level, higher taxonomic groups and total abundance for each sampling occasion were performed using univariate analysis. Comparisons were undertaken between test item treatment versus control and reference item versus control. Additionally, multivariate statistical methods were used to analyse effects on community level: Principal Component Analysis (PCA) and Principal Response Curves (PRC).

II. RESULTS AND DISCUSSION

A. FINDINGS

Results:

Multivariate analysis (PRC)
The applied reference item treatment gave reductions for a number of arthropod taxa. In the pitfall traps 3 out of 26 taxa analysed (including the total catch) were statistically significantly reduced. In the inventory samplings 8 of 29 taxa showed statistically significant reductions of the abundance. In the visual assessments no statistically significant effects of the reference item treatment on the one taxon evaluated were observed. The PRC confirmed a statistically significant influence of the reference item treatment on the arthropod community for the inventory sampling. For the pitfall traps the PRC was not statistically significant. Further the combined PRC (pitfall traps and inventory sampling) showed a statistically significant influence of the reference item treatment on the non-target arthropod community. However, more than 10 % of the taxa analysed were reduced by more than 50 % in all of the sampling methods used in the study. Therefore, the reference item proved that the test system was sensitive to the application of an insecticide.
According to the multivariate analysis (PRC) the test item treatment had a pronounced and long-lasting effect on the arthropod community caught by inventory sampling. Statistically significant differences to the control were observed directly after the last application until the end of the sampling period (7 to 124 DALA). If the most influential taxon Cicadellidae was excluded from the analysis then the last sampling was not statistically significant anymore and the effect lasted until 77 DALA. For the pitfall traps the PRC was not statistically significant. For the visual assessment no multivariate analysis was performed, due to a low number of taxa assessed.

Summary of pitfall trap sampling
The abundance of all arthropods caught in pitfall traps was not statistically significantly different in the test item treatment compared to the control during the sampling period. Numbers were comparable to control levels from the start of the study until the last sampling occasion (22 DBLA to 117 DALA).

Table B.9.5-35: Most abundant arthropods (n ≥ 72) caught with pitfall traps and statistically significant differences to the control

Taxon	**Life-stage**	**Sampling days after last application**					
		-22	7	35	56	77	117
Total catch		R					
Araneae total							
Linyphiidae total							
Erigone total							
Erigone atra	Adult	R#+					
Lycosidae total							
Lycosidae	Juvenile						
Trochosa ruricola	Adult				T		
Opiliones	Adult		T				
Myriapoda	Adult						
Coleoptera total							R+
Carabidae total							R+
Carabidae	Juvenile						
Amara total							
Amara aenea	Adult						
Nebria brevicollis	Adult			T			
Curculionidae	Adult						
Staphylinidae total					T+		
Diptera total							
Brachycera	Adult						
Nematocera	Adult						

Hymenoptera total		R					
Hymenoptera undet.	Adult						
Formicidae	Adult						
Aphididae (p)	Adult	T, R	T, R				
Collembola	Adult						

T: statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment (2 x 0.7 L Bulldock 25 EC/ha)
R = statistically significant decrease compared to the control ($p \leq 0.05$) of reference item.
+ behind T or R means statistically significant increased compared to the control ($p \leq 0.05$)
# behind T or R means statistically significant difference for the Wilcoxon-test ($p \leq 0.05$)
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Application 1 was performed on 07 June 2012, 14 days before application 2
Application 2 was performed on day 0 (21 June 2012)
(p) Pest species / Some species are considered as serious pest

4 taxa (Lycosidae species Trochosa ruricola, the order Opiliones, the Carabidae species *Nebria brevicolli* and the family Aphididae) showed a significant reduction and one taxa showed a significant increase (the family Staphylinidae) at a single sampling date after the two applications of Bulldock 25 EC (as: beta-cyfluthrin) in the test item treatment. All other 21 taxa were not affected. The result is not unexpected since the ground living arthropods are only affected by the drift rate of the test item.

Summary of visual assessment
The one taxon analysed, Aphididae, showed a statistically significant difference between the test item treatment and the control. This sampling type was the one with the lowest number of specimen in this study in terms of individual numbers and also of taxa caught. 16 assessments were performed. The first took place 8 days before the 1st application and the last 124 days after the 2nd application.
The aphids showed a statistically significant reduction at a single assessment date due to the two applications of Bulldock 25 EC (as: beta-cyfluthrin). Abundances of aphids (Aphididae, pest species) were below control level between the 2nd and the 6th sampling (10 DBLA to 29 DALA) with a significant reduction at the 3rd sampling (7 DALA). Afterwards numbers exceed control level until the 13th sampling (77 DALA). At the 13th sampling (77 DALA) numbers in the test item treatment were lower again compared to the control. The last three samplings exceed control level.

Summary of inventory sampling
The abundance of all arthropods caught by inventory sampling was statistically significantly lower in the test item treatment compared to the control at the 3rd and 4th sampling (35 and 57 DALA). The main trigger for this reduction was the order Auchenorrhyncha (cicadas and leaf hoppers), which showed a significant reduction at these sampling and accounted for approximately 20 % of all arthropods caught by inventory sampling. In the following samplings a recovery of arthropod abundance in the test item treatment was observed.

Table B.9.5-36: Most abundant arthropods (n ≥ 72) caught with inventory sampling and statistically significant differences to the control

Taxon	**Life-stage**	**Sampling days after last application**					
		-22	**7**	**35**	**56**	**77**	**117**
Total catch				T,R	T		
Araneae total			T+, R+	T			R
Clubionidae*	Juvenile			T		T	
Linyphiidae total			T+				
Linyphiidae	Juvenile		T+				
Theridiidae total							
Theridiidae	Juvenile						
Opiliones	Adult		T#, R	T#	T		
Coleoptera total						R	
Hydrophilidae	Adult						
Diptera total							
Cecidomyiidae	Adult						

Chironomidae	Adult						T
Chloropidae	Adult						
Dolichopodidae	Adult		T,R				
Drosophilidae	Adult						
Sciaridae	Adult						
Hymenoptera total				T			
Braconidae	Adult					T+	
Formicidae	Adult						
Ichneumonidae	Adult						
Platygasteridae	Adult						
Pteromalidae	Adult		T,R	T			
Auchenorrhyncha total(p)				T,R	T,R		T,R
Auchenorrhyncha(p)	Juvenile						
Cicadellidae (p)	Adult			T,R	T,R	T	T,R
Sternorrhyncha total							
Aphididae (p)	Adult						
Psyllidae*	Adult						

T = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment (2 x 0.7 L Bulldock 25 EC/ha)
R = statistically significant decrease compared to the control ($p \leq 0.05$) of reference item.
+ behind T or R means statistically significant increased compared to the control ($p \leq 0.05$)
# behind T or R means statistically significant difference for the Wilcoxon-test ($p \leq 0.05$)
* considered taxa due to Canoco analysis, but not matching the criteria $n \geq 72$
Empty cells mean no significant difference compared to the control ($p \leq 0.05$)
Application 1 was performed on 07 June 2012, 14 days before application 2
Application 2 was performed on day 0 (21 June 2012)
(p) Pest species / Some species are considered as serious pest

The order Opiliones (harvestmen) was present at low numbers at the beginning of the study (22 DBLA) in the control and the test item treatment. In contrast to the control abundances in the test item treatment did not increase, resulting in a statistically significant lower abundance at the 2nd, 3rd and 4th sampling (7, 35 and 57 DALA). This was most likely a direct effect of the applications of the test item. Until the end of the study abundances were on a comparable level to the control.
The abundance of the family Dolichopodidae (long-legged flies) was statistically significantly lower compared to the control at the 2nd sampling (7 DALA). This is most likely a treatment related effect. However, the effect was not long-lasting, as abundances approached control level in the following samplings and no further significant differences for the test item treatment were observed. The order Hymenoptera was statistically significantly reduced in the test item treatment compared to the control at the 3rd sampling (35 DALA). This effect might be treatment related. In the subsequent samplings numbers in the test item treatment were on control level again. The family Pteromalidae (parasitoid wasps) showed statistically significantly lower abundances for the 2nd and 3rd sampling (7 and 35 DALA). Since abundances in the control increased with normal population variability, the decreases in the test item treatment can be regarded as a direct effect of the application of the test item. An indirect effect due to the reduction of prey species might also play a role in the observed decrease of abundance. But despite the lower number of Cicadellidae (a possible prey species) throughout the study period numbers were comparable to the control in the last three samplings.
No specimens of the order Auchenorrhyncha (cicadas, leafhoppers and frog hoppers) were caught at the beginning of the study in the control and the test item treatment. Abundances in the control increased strongly until the 3rd sampling (35 DALA). In contrast abundances in the test item treatment did not increase to the same extent and therefore were statistically significantly lower at the 3rd, 4th and 6th sampling (35, 57 and 124 DALA). The test item application results in the chemical control of the order Auchenorrhyncha, which contains only sucking specimens. The reductions observed can be regarded as a direct effect of the test item. The statistically significant effects of total Auchenorrhyncha were mainly due to the reduction of the lower taxon within this order the family Cicadellidae (75.24 % of total Auchenorrhyncha), which was also statistically significantly affected at the 3rd, 4th, 5th and 6th sampling (35 to 124 DALA). Again the reason for the reduction is the feeding through sucking of specimens belonging to this family. Because of the late build-up of the cicada population in summer the effect was observed starting with the 3rd sampling end of July 2012.

III. CONCLUSIONS

The effect of Bulldock 25 EC applied twice to an apple orchard at 0.7 L product/ha (17.5 g beta-cyfluthrin/ha) is summarised below.

Pitfall traps:
Effects were only observed for single dates with exception of the family Aphididae caught by pitfall traps, which showed statistically significant differences for two dates. For Opiliones and the order Aphididae statistically significant effects occurred directly after the last application. This is most likely an effect caused by the applications of the test item. For the other three taxa (*Trochosa ruricola*, *Nebria brevicollis* and Staphylinidae) the effect can be regarded as chance or due to normal fluctuations in population numbers. Therefore, it can be concluded that ground-dwelling arthropods insects were only affected very short term and transiently (class 2; de Jong *et al.*, 2010).

Visual assessment:
Statistically significant effects of test item treatment were observed for the evaluated taxon during the study period. From the results it can be concluded that insects were only affected very short term and transiently (class 2; de Jong *et al.*, 2010).

Inventory sampling:
Abundances of the most taxa were comparable to the control at the end of the study, with the exception of cicadas. The statistically significant effects for cicadas lasted until the last sampling, 124 days after the last application when including this group in the PRC. This effect is very likely treatment related but considered acceptable since cicadas are a species targeted by the test item. Cicadas were by far the main contributors to the shape of the PRC analysis. Thus, when excluding cicadas (being target species) from the PRC, then statistically significant differences only lasted until 77 days after the last treatment.
The duration of the sampling in 2012 (approximately 5 months) was sufficient to demonstrate recovery for the community (excluding the family Cicadellidae).
Therefore, it can be concluded that non-target arthropods were affected by pronounced effects with a recovery within 4 months after the last application (class 4; de Jong et al., 2010).

Reliablity:

However, the study was conducted in orchard. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis.
The following orders are lacking completely:

1. Lepidoptera
2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera
6. Dermaptera

The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

Consequently, the study is classified as not reliable R3 (according de Jong, 2010).

KIIIA1 10.5.3/07 (newly submitted with the dossier)

Author:	Mack P.
Title:	A Field Study Assessing the Impact of Drift Rates of beta-cyfluthrin on the Non-Target Arthropod Fauna on a Meadow in Germany
Date:	13 March 2014
Doc ID:	
Report no.:	S13-00176
Edition no.:	R-30607
Guidelines:	Candolfi *et al.* (2000), de Jong *et al.* (2010)
GLP:	yes
Validity:	R3 (not reliable) according de Jong et al. 2010

Summary:

In this study potential effects of the test item on arthropod population density and composition of the sampled communities throughout the study period were monitored.
The study was conducted on a meadow in Ofterdingen, Germany. A meadow was selected because it was considered to be representative for off-crop areas. Six treatment groups were set up: control (tap water), four rates of the test item (Bulldock 25 EC) and a reference item (Danadim Progress; dimethoate 400 g/L). Bulldock 25 EC was applied once at nominal rates of 4 mL/ha, 16 mL/ha, 64 mL/ha and 240 mL/ha (nominally 0.1 g as/ha, 0.4 g as/ha, 1.6 g as/ha and 6.0 g as/ha) for test item treatments T1, T2, T3 and T4, respectively. All treatments comprised four plots (replicates) of about 900 m^2 each.
The reference item (Danadim Progress) was applied once at a nominal rate of 4 L/ha. The reference item was used to confirm the sensitivity of the test system. It was chosen because of its known broad insecticidal effects on arthropods. All plots were sprayed with a calibrated boom sprayer. The control plots were sprayed first with tap water, followed by the four test item groups with ascending rates, and finally the application of the reference item plots was performed. The population development and species composition of plant-(foliage) dwelling and ground livingarthropods (e.g. insects and spiders) were monitored in all treatment groups. Population monitoring was carried out using Pitfall traps, Photoeclectors and Vortis suction sampling. To determine the initial population levels, each sampling method was performed once before the application. Additionally a Visual Assessments of the vegetation in the four blocks was conducted. The effect of the test item treatment was assessed by comparing the data for the arthropod populations in the plots treated with the test item to the populations in the water treated control plots.

Results : NOEC $_{population}$ < 0.1 g as/ha
According de Jong (2010) the study is classified as R3. Therefore, it is considered not appropriate for assessing the risk of NTAs in off-field areas.

I. MATERIALS AND METHODS

MATERIAL

1. Test material:

Test item: Bulldock 25 EC
Description: Liquid / yellow
Lot/Batch #: 92110164
Purity: 25 g/L (nominal), 24.79 g/L (measured)

2. Vehicle and/or positive control:

Control: water sprayed
Reference item: Danadim Progress: 400 g/L dimethoate

B: STUDY DESIGN AND METHODS

1. Location, description of test site

The field study was performed on a meadow in Ofterdingen, Baden-Württemberg, Germany.
Description of the field site (copied from the study report)

Location	Ofterdingen
ZIP code	72131
Federal state / Region	Baden-Württemberg
Country	Germany
Longitude/Latitude	Long: 9.005462°E ; Lat: 48.407625° N
Sea level	480 m
Slope	2 %

Table B.9.5-37: Field site history and maintenance (2010 – 2013) (copied from the study report)

Field crop history	
2010	Meadow
2011	Meadow
2012	Meadow
2013	Meadow
Fertilisation	
24 Mar 2010	NPK (nitrogen, phosphorus and potassium); 250 kg/ha
05 Jul 2010	ammonium nitrate solution; 220 kg/ha
20 Apr 2011	NPK (nitrogen, phosphorus and potassium); 230 kg/ha
06 Jun 2011	ammonium nitrate solution; 220 kg/ha
27 Mar 2012	NPK (nitrogen, phosphorus and potassium); 250 kg/ha
20 Jun 2012	ammonium nitrate solution; 220 kg/ha
23 Apr 2013	NPK (nitrogen, phosphorus and potassium); 134 kg/ha
24 Jun 2013	ammonium nitrate solution; 220 kg/ha
2010	3 x mowing
2011	4 x mowing
2012	2 x mowing
2013	2 x mowing (16 DBA, 43DAA)

Table B.9.5-38: Soil characterisation results (copied from the study report)

Parameters			
Soil type (USDA) [%]	[%]	sand	3.4
		Silt	14.7
		clay	81.9
pH (H2O)			6.6
pH (CaCl2)			5.5
Total carbon	[%]		9.7
Total organic carbon	[%]		8.9
Soil type			clay

Vegetation:
At the visual assessment of vegetation (15 DAA) 7 grasses and 26 dicotyledon species were recorded in the four blocks. The most abundant species (with a mean over the four blocks of > 10 %) occurring in all blocks, were *Poa annua* (annual meadow grass, 17.84 %) and *Holcus lanatu*s (velvet grass, 17.53 %;). Thus, the field site can be classified as a cultivated pasture (Molinio-Arrhenatheretum).

Table B.9.5-39: Sampling scheme including weather conditions (summaried according the research report)

Date	Activity	Sample type	Days after application	Air /soil temperature [C°]	Air humidity [%]	Cloud cover [%]
28 Jun 2013		VCS	-3	15.0-19.6/18.2	44.8 – 50.3	60
29 Jun 2013		PFT, PE	-2	13.0-14.6 / 15.7	78.6-96.1	100
1 Jul 2013	Application					
5 Jul 2013		VCS	4	21.2-29.5/23.1	38.8-62.2	70
6 Jul 2013		PFT, PE	5	20.7-28.5/17.5	46.8-76.7	0
9 Jul 2013		VCS	8	25.7-30.1/21.8	33.1-57.0	50
11 Jul 2013		PFT, PE	10	17.7-25.4/21	49.5-79.9	80
15 Jul 2013		VCS	14	24.8-26.5/20.0	29.6 – 58.6	10
20 Jul 2013		PFT, PE	19	24.4-32.3/23.4	29.6-58.6	10
22 Jul 2013		VCS	21	30.8-32.8/22.3	27.6-38.4	5
27 Jul 2013		PFT, PE	26	28.8-35.4/25.5	35.6-63.4	30
1 Aug 2013		VCS	31	30.3-31.8/24.0	24.8-48.5	0
6 Aug 2013		PFT, PE	36	24.6-28.0/22.8	56.7-75.4	20

Test design, application, concentrations and replicates

The field study was carried out on a meadow in Ofterdingen, Germany, and was in compliance with the ‘Guideline forRegulatory Field Testing’ (CANDOLFI et al., 2000) and the ‘Guidance for summarising and evaluating field studies with nontarget arthropods’ (DEJONG et al., 2010). The study
consisted of one field trial, S13-00176-01, and one taxonomic phase, S13-00176-02 for Pitfall traps, Photoeclectors and Vortis suction samplings.
The first sampling was 2 to 3 days before treatment and the final sampling was approximately one month after treatment.
Three different sampling methods were used: Pitfall traps, Photoeclectors and Vortis suction sampling. Pitfall trap, Photoeclector and Vortis suction samplings were performed six times, each.
At the beginning of the sampling period a visual assessment of vegetation was performed.
The trial included four test item groups with Bulldock 25 EC (T1, T2, T3, T4), a water treated control and a reference item treatment (Danadim Progress) with one application each to assess the sensitivity of the test system. All treatments comprised four plots (replicates) of about 900 m2 each.

C = tap water treated control
T1 = Bulldock 25 EC (4 mL product/ha; 0.1 g as/ha nominal)

T2 = Bulldock 25 EC (16 mL product/ha; 0.4 g as/ha nominal)
T3 = Bulldock 25 EC (64 mL product/ha; 1.6 g as/ha nominal)
T4 = Bulldock 25 EC (240 mL product/ha; 6.0 g as/ha nominal)
R = Danadim Progress (dimethoate 400 g/L;4 L product/ha)

All applications were conducted with a spray volume of 100 L water/ha.

The applications of the test item, of the reference item and the control water were carried out with a calibrated boom sprayer (Schachtner, 6 m boom, 12 nozzles).

Verification of rates

Before the applications the sprayer was calibrated and according to the output the duration of spraying per plot was calculated. Before the calibration the nozzles were visually checked and blocked nozzles were replaced. For the calibration, the individual nozzle outputs of the 12 nozzles were measured in three runs (each run lasted 60 s). The output of the single nozzles as well as the mean nozzle output of each run had to be within a limit of 5 % of the nominal volume. Exact data of the application and calibration were recorded

The actual amount of the test item (Bulldock 25 EC) and the reference item (Danadim Progress) applied was determined by recording the amount of spray solution prepared and the amount remaining after the application of each plot. Actual applied spray volume for the test item treatments and the reference item treatment were within the spray tolerance of □10 % for all plots.

Test conditions
Meteorological data were obtained from the nearest weather station (1km). On day of the application (01 Jul 2013) the weather was sunny with temperatures during the application of the control, test item treatments and reference item treatment between 16.8 °C and 29.8 °C.

The climatic conditions during the trial compared to the long-term average (1961-1990) revealed slightly higher average temperatures in June, July and August compared to the long-term average. The rainfall at the field site was about 74 % in June 2013, 127 % in July 2013 and 119 % of the long-term average in August 2013, recorded at a weather station approximately 3 km distance from the field site.

Sampling
The sampling scheme is shown in Table B.9.5-39.

Vortis suction sampling gives an indication of arthropod abundance on the sampled area. Suction samplers have been chosen for studies on ground dwellers such as spiders, springtails, ants and beetles in grassland (e.g. TÖRMALA 1982).
Although efficiency is always less than 100 %, suction samples represent estimates of population levels per unit area and thus allow statistical comparisons between treatments for the same habitat and time. In addition, foliage-dwelling arthropods are sampled with Vortis suction sampling.
Vortis suction sampling is biased towards smaller arthropods. Large beetles or larger spiders are under-represented.

Pitfall traps have been extensively used for studies on surface dwellers such as spiders, springtails, centipedes, ants and beetles, especially ground beetles (e.g. CLEMENTS *et al.*, 1988; EDWARDS *et al.*, 1979; GREENSLADE, 1964). Whilst
Pitfall traps do not estimate actual populations but a combination of activity/abundance (BRIGGS, 1961; LUFF, 1975), their use is appropriate and routine in comparative field studies.

Photoeclectors are used to sample positively photo-tactic arthropods emerging from the soil (e.g. adult insects emerging from pupae, or larvae, or overwintered adults), ground-dwelling arthropods and phytophagous arthropods.

II RESULTS AND CONCLUSION

FINDINGS

In total **106,143** arthropods were caught in this study and identified.

Reference item
The applied reference item treatment (applied on the same day as the test item; Danadim Progress (dimethoate 400 g/L) at a rate of 4 L/ha, equivalent to 1600 g as/ha) gave a reduction for a number of arthropods and a change in diversity of the community for all sampling types. In the Pitfall traps of the 27 taxa analysed eighteen were statistically significantly reduced. Statistically significantly increased abundances were observed for one taxon. In the Photoeclector assessments, of the 23 taxa analysed, nine taxa were statistically significantly reduced. A statistically significant increase in abundance was observed for one taxon. In the Vortis suction samples of the twenty analysed taxa fifteen showed a statistically significantly reduced abundance due to the application of the reference item. The validity criterion for the reference treatment was clearly met (at least 50 % reduction on at least one sampling date for more than 50 % of the taxa evaluated).
The PRCs confirmed a statistically significant influence of the reference item treatment for the Pitfall traps, the Photoeclectors and the Vortis suction sampling on the arthropod community. Therefore, the reference substance proved that the test system was sensitive to the application of an insecticide.

Multivariate analysis (PRC)
According to the multivariate analysis (PRC) the four test item treatments had no impact on the ground and crop-dwelling arthropod communities collected with Photoeclector sampling. For the Pitfall traps treatment related effects on the community composition were observed during the study period for test item treatment T3 at the 3rd and 5th sampling and for test item treatment T4 at the 2nd, 3rd and 5th sampling.
For Vortis suction sampling data a statistically significant impact of test item treatment T3 was observed at the 2nd and 3rd sampling and of test item treatment T4 from the 2nd to the 5th sampling. Most of the variation was based on the population dynamics due to seasonal changes, causing fluctuations in species composition of communities. For data from all three sampling methods, the multivariate Principle
Response Curve (PRC) analysis revealed that 84.0 - 86.7 % of the total variation was not related to treatment but was either due to time (seasonal changes) or can be classed as random. 13.3 - 16.0 % of the variation was treatment related. The combined community response of the Pitfall traps, Photoeclectors and Vortis suction sampling showed a statistically significant impact of test item treatments T1 at the 5th sampling, of test item treatment T3 at the 3rd sampling and of test item treatment T4 at the 2nd, 3rd and 5th sampling. The significant effect of test item treatment T1 is not considered treatment related as none of the single sampling type PRC's revealed a significant effect of test item treatments T1 and T2. No statistically significant differences were observed in earlier and later samplings. Test item treatment T2 showed a statistically significant difference at the 1st sampling before the application of the test item. This might be caused by the Pitfall trap data, where the same difference was observed for test item treatment T2.
However, no statistically significant effect on the community composition after application of the test item treatment T2 occurred. Therefore no effect of the test item treatment T2 could be found. This combined analysis is considered relevant due to overlap of species sampled by each method.

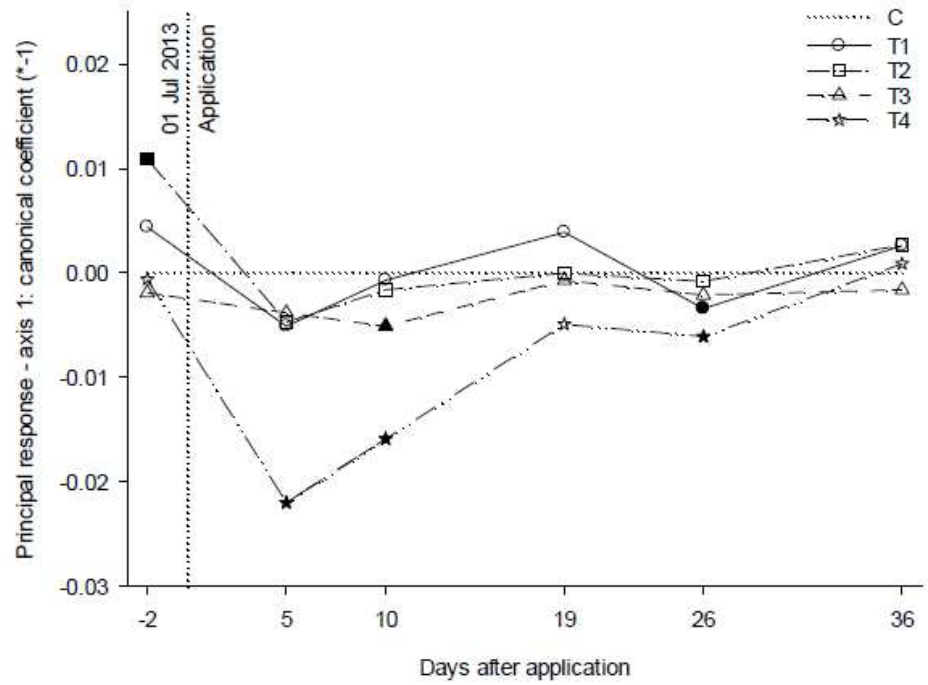

Figure 9.5-1: Principal Response Curve for the community of ground- and crop-dwelling arthropods of Pitfall traps, Photoeclectors and Vortis suction sampling combined for test item treatments (Canonical Coefficient); axis 1

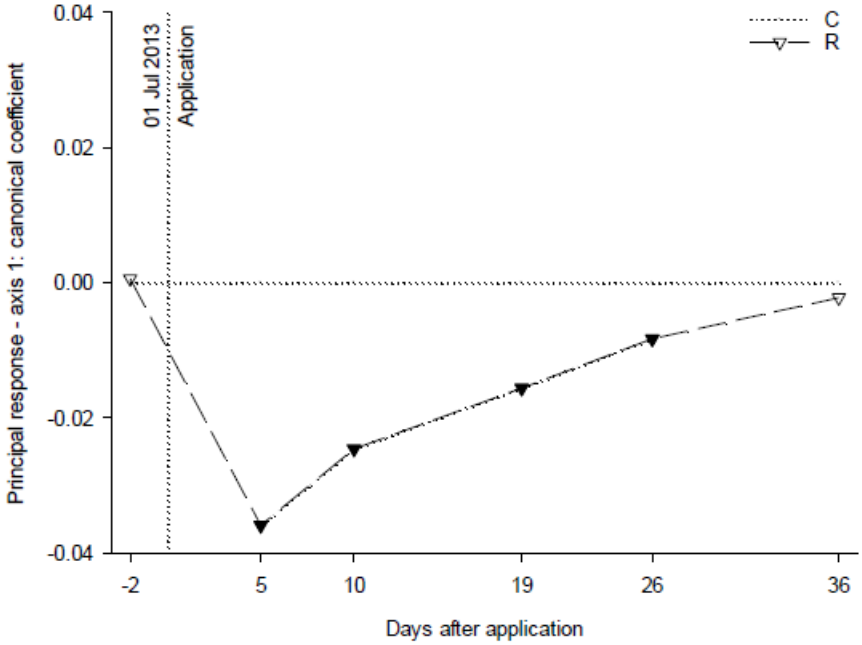

Figure 9.5-2: Principal Response Curve for the community of ground- and crop-dwelling arthropods of Pitfall traps, Photoeclectors and Vortis suction sampling combined for reference item treatment (Canonical Coefficient); axis 1

Univariate analysis

The results for the univariate statistics are discussed in the following parts, since the results are much more detailed.

Taxonomy

The counting and taxonomic identification of the arthropods collected were performed by BTL Bio-Test Labor GmbH, Groß Lüsewitz, Germany (Pitfall traps, Photoeclectors, Vortis suction sampling). After receipt, the samples were stored at BTL and the labels were checked for conformity with the sample codes in the study plan.
Taxonomic resolution depended somewhat on the sampling method. The highest level of resolution was used for the most relevant sampling method(s) for each taxon. The taxonomic identification was done as follows:
For the Pitfall traps Araneae, Carabidae, Coccinellidae and Staphynilidae were identified to species level. Other Coleoptera, Neuroptera, Sternorrhyncha, Auchenorrhyncha, and Formicidae were identified to families. Dipterans were identified to the two suborders Brachycera and Nematocera and were split into juveniles and adults. Opiliones, Dermaptera, Orthoptera, Hymenoptera and Isopoda were identified to the order. Specimens of the sub-phylum Myriapoda were counted only.
For the Photoeclectors identification was performed for the following taxa sampled: Araneae, Carabidae, Coccinellidae and Staphylinidae were identified to species level. Diptera were identified to sub-order and split into adults and juveniles. Sternorrhyncha, Neuroptera, Hymenoptera, Auchenorrhyncha and phytophagous Coleopterans were identified to family level. Opiliones, Lepidoptera, Dermaptera, Orthoptera and Isopoda were counted only. Specimens of the sub-phylum Myriapoda were counted only.
For the Vortis suction sampling identification was performed for the following taxa sampled: Araneae, Carabidae, Coccinellidae and Staphylinidae were identified to species level. Sternorrhyncha, Hymenoptera, Auchenorrhyncha, Neuroptera and other Coleopterans were identified to family level. Dipterans were identified to the suborder and were split into larvae and adults. Opiliones, Orthoptera, Dermaptera, Lepidoptera, Psocoptera and Isopoda were counted only. Specimens of the sub-phylum Myriapoda were counted only.

Pitfall Traps

In total 51,445 arthropods were caught in 6 sampling periods with Pitfall traps during the study period. 10,109 were caught in the control plots. Two taxa were collected in dominant abundances (*Lycosidae juvenile and Brachycera adult*), six taxa were subdominant (*Erigone atra* adult, *Pardosa palustris* adult, *Poecilus cupreus* adult, Nematocera adult, Hymenoptera adult, Cicadellidae adult) and fourteen taxa were receding (Linyphiidae others, *Meioneta rurestris* adult, *Pardosa pullata* adult, *Pachygnatha degeeri* adult, *Amara aenea* adult, *Amara similata* adult, *Anisodactylus binotatus* adult, *Carabus monilis* adult, *Pseudophonus rufipes* adult, Chrysomelidae adult, Aleocharinae adult, Staphylinidae others adult, Dermaptera adult and Aphididae adult).
23 taxa fulfilled the criterion of a mean number ≥ 1 for each plot of the control throughout the growing season (at least 96 specimens over all samplings in the control) and were included in the statistical analysis. The juvenile Tetragnathidae (Araneae) and the juvenile Carabidae (Coleoptera) were included in the statistical analysis because of the importance of their families and as representatives of the juvenile stage, though abundances were < 96 specimens over all samplings in the control. Additionally the spider family Linyphiidae (others) and the beetle family Staphylinidae (others), respectively, were summarised and evaluated as single taxonomical groups with > 96 specimens over all samplings in the control.

The results for the individual taxa analysed are presented in the following table:

Table B.9.5-40: Most abundant arthropods (n ≥ 96) caught with Pitfall traps in the control and their

statistically significant differences to the control

Taxa	Life-stage	Sampling					
		2 DBA	5 DAA	10 DAA	19 DAA	26 DAA	36 DAA
Linyphiidae (others)							
Erigone atra	adult			T4, R	R	R	
Meioneta rurestris	adult		T1, T3, R#	T2, T3, T4, R	R#	R	
Lycosidae	juvenile			T4			R
Pardosa palustris	adult		T2, T3, T4, R	T3, T4, R	T4, R	T4, R	
Pardosa pullata	adult		R	T4, R	R		
Trochosa ruricola	adult		T4, R			T3	
Tetragnathidae*	juvenile						R#
Pachygnatha degeeri	adult		T2+, R	T3, T4, R		T4	
Carabidae*	juvenile						
Amara aenea	adult	T4	R#				
Amara similata	adult		T3, T4, R			T2, R	
Anisodactylus binotatus	adult		R	R			
Carabus monilis	adult		T3, T4, R		T2, T3, R		
Poecilus cupreus	adult		R	R			
Pseudophonus rufipes	adult		R	R#			
Pterostichus melanarius	adult						
Pterostichus vernalis	adult		R			T3+, T4+	
Chrysomelidae	adult		T3, T4, R	T3, T4	T3		
Aleocharinae	adult	T1, T2				R#	
Staphylinidae (others)					R		
Brachycera	adult		T3+, T4+, R+	T4			T1
Nematocera	adult						
Dermaptera	adult			R		R	
Hymenoptera	adult		R				
Cicadellidae	adult		T2+, T4+	T4	T3, T4, R	T3, T4, R	T4, R#
Aphididae	adult						

T1 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T1 (0.1 g as/ha)
T2 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T2 (0.4 g as/ha)
T3 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T3 (1.6 g as/ha)
T4 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T4 (6.0 g as/ha)
R = statistically significant decrease compared to the control ($p \leq 0.05$) of reference item treatment.
+ behind T1, T2, T3, T4 or R means statistically significant increase compared to the control ($p \leq 0.05$)

Wilcoxon test ($p \leq 0.05$)
* < 96 specimens over all samplings in the control
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Application was performed on day 0, DBA = days before application, DAA = days after application

Photoeclector
In total 18,769 arthropods were caught on 6 sampling occasions with Photoeclector sampling during the study period. 3,369 were caught in the control plots. One taxon was collected in **eudominant abundances** (*Brachycera adult*), one taxon was **dominant** (*adult Nematocera*), three taxa were **subdominant** (Formicidae adult, Platygasteridae adult and Scelionidae adult) and twelve taxa were **receding** (total Linyphiidae, Lycosidae juvenile, Tetragnathidae juvenile, Carabidae others, Chrysomelidae adult, Aleocharinae adult, Staphylinidae others, Braconidae adult, Ceraphronidae adult, Eucoilidae adult, Cicadellidae adult and Aphididae adult).
22 taxa fulfilled the criterion of a mean number ≥ 1 for each plot of the control throughout the growing season (at least 24 specimens over all samplings in the control) and were included in the statistical analysis. Additionally the beetle family Carabidae (others) and the beetle family Staphylinidae (others), were summarised and evaluated as single taxonomical groups with > 24 specimens over all samplings in the control.

The results for the individual taxa analysed are presented in the following table:

Table B.9.5-41: Most abundant arthropods (n ≥ 24) caught with Photoeclectors sampling in the control and their statistically significant differences to the control

Taxa	**Life-stage**	**Sampling**					
		2 DBA	**5 DAA**	**10 DAA**	**19 DAA**	**26 DAA**	**36 DAA**
Linyphiidae total							
Lycosidae	juvenile		T1, T2, T3, T4, R				R
Tetragnathidae	juvenile						
Carabidae (others than *Pterostichus*)		R				T1+	
Pterostichus total		T2+, T4+					
Chrysomelidae	adult						
Aleocharinae	adult	R					R+
Staphylinidae (others than *Philonthus*)							
Philonthus cognatus	adult					T3, T4	
Brachycera	adult			R			
Nematocera	adult						
Braconidae	adult						
Ceraphronidae	adult						
Diapriidae	adult						
Eucoilidae	adult		R	T1+, T4+			
Eulophidae	adult						
Formicidae	adult						
Mymaridae	adult				T3, R	R	R
Platygasteridae	adult		R				
Scelionidae	adult			R	R		
Cicadellidae	adult		T1+	T3, T4, R#		R	R
Delphacidae	adult			T2	R		
Aphididae	adult			R#			

T1 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T1 (0.1 g as/ha)
T2 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T2 (0.4 g as/ha)
T3 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T3 (1.6 g as/ha)
T4 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T4 (6.0 g as/ha)
R = statistically significant decrease compared to the control ($p \leq 0.05$) of reference item treatment.
+ behind T1, T2, T3, T4 or R means statistically significant increase compared to the control ($p \leq 0.05$)
# Wilcoxon test ($p \leq 0.05$)
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Application was performed on day 0, DBA = days before application, DAA = days after application

Vortis suction sampling

In total 35,929 arthropods were caught from the 6 sampling occasions with Vortis suction sampling during the study. 7,395 specimens were caught and counted in the control plots. Two taxa were collected in dominant abundances (Brachycera adult and Aphididae adult), six taxa were subdominant (Tetragnathidae juvenile, Chrysomelidae adult, Nematocera adult, Braconidaeadult, Cecadellidae adult and Delphacidae adult) and nine taxa were receding (Linyphiidae juvenile, Apionidae adult, Aleocharinae adult, Ceraphronidae adult, Eulophidae adult, Formicidae adult, Mymaridae adult, Platygasteridae adult and Pteromalidae adult).
18 taxa fulfilled the criterion of a mean number ≥ 1 for each plot of the control throughout the growing season (at least 48 specimens over all samplings in the control) and were included in the statistical analysis. Due to the sensitivity of juvenile spiders the juvenile Lycosidae were additionally added to the statistical analysis, although abundances were < 48 specimens over all samplings in the control. Further the beetle family Staphylinidae (others) was summarised and evaluated as single taxonomical groups with > 48 specimens over all samplings in the control.

The results for the individual taxa analysed are presented in the following table:

Table B.9.5-42: Most abundant arthropods (n ≥ 48) caught with Vortis suction sampling in the control and their statistically significant differences to the control

Taxa	**Life-stage**	**Sampling**					
		3 DBA	**4 DAA**	**8 DAA**	**14 DAA**	**21 DAA**	**31 DAA**
Linyphiidae	juvenile			T1+			R
Lycosidae *	juvenile			R			
Tetragnathidae	juvenile		R	R	R	R	R
Apionidae	adult						
Chrysomelidae	adult		T4, R#	T4, R	R		
Staphylinidae (others than Aleocharinae)			R				
Aleocharinae	adult		R	T1+, T2+, T3+, T4+			
Brachycera	adult			T4, R	R		
Nematocera	adult		R	T4	T4	T4	
Braconidae	adult		R	R			
Ceraphronidae	adult						
Eulophidae	adult	T3	R	R			
Formicidae	adult				T1		
Mymaridae	adult		R	R		R	
Platygasteridae	adult			T4, R	R		
Pteromalidae	adult			R			
Scelionidae	adult		R				
Cicadellidae	adult		T3, T4, R	T3, T4, R	T4, R	T4, R	T3, T4, R
Delphacidae	adult		T3, R	R	R		
Aphididae	adult		R	R	R		

T1 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T1 (0.1 g as/ha)
T2 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T2 (0.4 g as/ha)
T3 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T3 (1.6 g as/ha)
T4 = statistically significant decrease compared to the control (t-test, $p \leq 0.05$) of test item treatment T4 (6.0 g as/ha)
R = statistically significant decrease compared to the control ($p \leq 0.05$) of reference item treatment.
+ behind T1, T2, T3, T4 or R means statistically significant increase compared to the control ($p \leq 0.05$)
# Wilcoxon test ($p \leq 0.05$)
* < 48 specimens over all samplings in the control
Empty cells mean no statistically significant difference compared to the control ($p \leq 0.05$)
Application was performed on day 0, DBA = days before application, DAA = days after application

Table B.9.5-43: Summary table effect classifications

Effect classification (based on De Jong *et. al.*, 2010):		**Effect class:**
one occasion	Slight and transient effects observed on one occasion only	**2**
< 2 months (a)	Effects no longer statistically significant on the last two sampling dates	**3a**
< 2 months (b)	Effects no longer statistically significant on the last sampling date	**3b**
> 2 months	Pronounced effects; no recovery within the study period	**8**

Table B.9.5-44: Summary of community level effects

Community level effects	**Treatment**			
	T1	**T2**	**T3**	**T4**
(PRC/Monte-Carlo; 5 % alpha level)	**Effect class**			
Pitfall traps	-	-	**3b**	**3b**
Photoeclectors	-	-	**-**	**-**
Vortis suction sampling	-	-	**3a**	**3b**
Combined dataset (Pitfall traps, Photoeclectors and Vortis suction sampling)	-	-	**2**	**3b**

T1 = 0.1 g as/ha; T2 = 0.4 g as/ha; T3 = 1.6 g as/ha; T4 = 6.0 g as/ha#

Table B.9.5-45: Summary table of population level effects

Population level effects			**Treatment**			
Sampling type	**Taxa**	**Lifestage**	**T1**	**T2**	**T3**	**T4**
Effect class						
PT	*Erigone atra*	adult	-	-	-	2↓
PT	*Meioneta rurestris*	adult	2↓	2↓	3a↓	2↓
PT	**Lycosidae**	juvenile	-	-	-	2↓
PT	*Pardosa palustris*	adult	-	2↓	3a↓	3b↓
PT	*Pardosa pullata*	adult	-	-	-	2↓
PT	*Trochosa ruricola*	adult	-	-	2↓*	2↓
PT	*Pachygnatha degeeri*	adult	-	2↓*	2↓	3b↓
PT	*Amara similata*	adult	-	2↓*	2↓	2↓

PT	*Carabus monilis*	adult	-	2↓*	3a↓	2↓
PT	*Pterostichus vernalis*	adult	-	-	2↑*	2↑*
PT	**Chrysomelidae**	adult	-	-	3a↓	3a↓
PT	**Brachycera**	adult	2↓*	-	2↑	3a↑↓
PT	**Cicadellidae**	adult	-	2↑*	3b↓	8↓
PE	**Lycosidae**	juvenile	2↓*	2↓*	2↓*	2↓
PE	Carabidae others		2↑*	-	-	-
PE	*Philonthus cognatus*	adult	-	-	2↓*	2↓*
PE	**Eucoilidae**	adult	2↑*	-	-	2↑*
PE	**Mymaridae**	adult	-	-	2↓*	-
PE	**Cicadellidae**	adult	2↑*	-	2↓	2↓
PE	**Delphacidae**	adult	-	2↓*	-	-
V	**Linyphiidae**	juvenile	2↑*	-	-	-
V	**Chrysomelidae**	adult	-	-	-	3a↓
V	Aleocharinae	adult	2↑*	2↑*	2↑*	2↑*
V	**Brachycera**	adult	-	-	-	2↓
V	**Nematocera**	adult	-	-	-	3b↓
V	**Formicidae**	adult	2↓*	-	-	-
V	**Platygasteridae**	adult	-	-	-	2↓
V	**Cicadellidae**	adult	-	-	8↓	8↓
V	**Delphacidae**	adult	-	-	2↓*	-

* Effect not treatment related
- No consistent statistically significant adverse effect observed
T1 = 0.1 g as/ha; T2 = 0.4 g as/ha; T3 = 1.6 g as/ha; T4 = 6.0 g as/ha
PT = Pitfall trap sampling, PE = Photoeclector sampling, V = Vortis suction sampling

CONCLUSION

No statistically significant adverse community effects were found up to and including the rate 0.4 g as/ha. This rate is classified as the community NOER (No Observed Effect Rate).
The rate 6.0 g as/ha is the community NOEAER (No Observed Ecologically Adverse Effect Rate). At this rate statistically significant adverse community effects were observed with recovery until the end of the study period.
However, endpoints based on community level are not regarded appropriate for assessing the risk of non-target arthropods.
At the population level several taxa were considered adversely affected by treatment with beta-cyfluthrin at the rates 1.6 g as/ha and 6.0 g as/ha. For both rates, respectively one taxon (Cicadellidae) did not recover within the sampling period.

For test item treatments 0.4 g as/ha statistically significant adverse population effects were observed with recovery until the end of the study period.
Though, due to the arrangement of study plots and the open – air design, it is not possible to distinguish between recovery and recolonialisation. Therefore, recolonialisation has to be assumed. As the recolonialisation of the in-field occurs from the off-field, endpoints including recolonialisation can not be accepted for off-field areas.

It's stated that class 2 effects to *Meioneta rurestris* for treatment T1 are not treatment related.
The statistically significant reduction at the 2nd sampling in test item treatment T1 is unlikely to be

treatment related as numbers were very low at the beginning of the study and are most likely due to natural fluctuations in dispersal. In the 3rd sampling abundances in the control increased to a level where significances can be reliably interpreted as treatment related.

However, the low abundance before the treatment start can be observed for more than one species and is generally regarded as one reason for the lacking reliability of the study. Thus, statistically significant effects have to be considered as treatment related especially if the same effects proceed in the following treatments. **Thus, the $NOEC_{population}$ is < T1**.

Classification of reliability:
The study is **classified as R3 (not reliable)** according de Jong et al. 2010:

1. Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis. In Contrast arthropods sampled in this study belong to only 22 different families (out of 6 orders) . The following orders are lacking completely:
    1. Collembola (above-ground)
    2. Lepidoptera
    3. Neuroptera
    4. Orthoptera
    5. Psocoptera
    6. Thysanoptera

2. The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

3. The abundance of the sampled (statistically evaluated) taxa few days (-2 and -3 days) before treatment is very low for several species and rises during the study course (especially in the control). Therefore possible short-term effects right after application (typical for knock-out effects usually caused by pyrethroids) might stay undetectable.

Several reasons for the low general diversity in the plots as well as for the relatively low abundance of sampled taxa before treatment start might be discussed.
Besides changing weather conditions from the first sampling time (average air temperatures: 13-14.6 °C) to the second sampling time (average air temperatures: 21.2 - 29.5 °C), the mowing of the treatment plots 16 days as well as the fertilisation 8 days before treatment are conceivable causes. The latter two are not comprehensible and could have been avoided. Furthermore, agricultural measures are not in line with natural off-crops (like natural meadows).

3. The plot design is very unpropitious. For this reason it cannot be distinguished between recolonisation and real recovery. Only the latter appears to be acceptable for risk assessment of the off-crop areas:

1. Plots are regarded as too small (0.09 ha/plot). In contrast de Jong et al. (2010) recommends a minimum plot area of 1 ha.
2. The distance between plots of 10 m is regarded as too close to exclude a recolonisation from less exposed plots and/or control plots to higher treated plots instead of a real recovery.
3. In general, closed plots (cage) should be favored to exclude recolonisation.

4. Generally, the RMS is of the opinion, that arthropod communities of grassland are not representative for all off-crop areas in Europe. Thus, it’s regarded impossible to cover the risk of pesticides to non-target arthropods with one off-field study conducted in one type of habitat.

B.9.6 Risk assessment for arthropods

Sensitive indicator species

After evaluating/ re-evaluating all available laboratory studies (Tier1) and extended laboratory studies (Tier 2) must of them turned out to be invalid and/or not plausible

The following studies are considered valid and appropriate for risk assessment:

Typhlodromus pyri	LR50 = 0.0025 g as/ha 20 %, 28 %, 41 %, 82 % and 100 % mortality at 0.3, 0.9, 2.7, 8.1 and 24.3 mg as/ha		KIIIA1 10.5.1/02 FC010TPL Roig, 2014a M-479587-02-1 R-33356	valid for assessing mortality; effects on reproduction were not investigated
Aphidius rhopalosiphi	LR50 = 0.163 g as/ha 3 %, 5 %, 25 %, 29 % and 90 % mortality at 20, 40, 80, 160 and 320 mg as/ha		KIIIA1 10.5.1/01 FC011ARL Roig, 2014b M-479582-01-1 R-33355	valid for assessing mortality; effects on reproduction were not investigated
Poecilus cupreus	7.7 g as/ha: Mortality: 0 % Slight effects on food consumption up to 2 days afterapplication		KIIIA1 10.5.1/01 HBF/CA 27 Heimbach, 1990 M-052707-01-1 R-19124	valid
Poecilus cupreus, larvae	Bulldock EC 025 Extended Lab., formulation mixed into soil	LR_{50}> 0.04 mg as/kg soil $LR_{100} \leq$ 0.4 mg as/kg soil NOEC < 0.04 mg/as kg/soil	KIIIA1 10.5.2./02 Neumann (2001) M-080415-01-1	valid

Based on the determination of mortality, the newly submitted laboratory tests with *T.pyri* and *A. rhopalosiphi* reveal a very high toxicity of the representative product Bulldock EC25 on both species. However, effects on reproduction were not investigated. Therefore a final statement about the level of toxicity cannot be made.

Based on valid tier 1 data, the most sensitive endpoint for leaf dwelling arthropods is the LR_{50} = 0.0025 g ai/ha (derived by the standard laboratory study with *T. pyri*).

In case of soil-dwelling arthropods larvae of *Poecilus cupreus* turned out to be more sensitive than adult beetles.

B.9.6.1 Exposure

In-field exposure

The in-field exposure, given as predicted environmental rates, PER, for non-target arthropods resulting from the intended uses of Bulldock EC 25 is calculated according to published agreement after ESCORT 2 workshop (Candolfi et al. 2001[1] -hereafter referred to as 'Guidance Document') using the

[1] Candolfi, M.P.; Barrett, K.L.; Campbell, P.; Forster, R.; Grandy, N.; Huet, M.C.; Lewis. G.; Oomen, P.A.; Schmuck, R.; Vogt, H. (2001): Guidance document on regulatory testing and risk assessment prcedures for plant protection products with non-target arthropods. ESCORT2 Workshop European

following equation:

$$PER_{in-field} = \text{Application rate (g a.s./ha)} \times \text{MAF}$$

where:

MAF = generic multiple application factor used to take into account the potential build-up of applied substances between applications. This factor integrates number of applications, application interval and degradation kinetics of the active substance
Default MAF values for given numbers of applications are listed in the Guidance Document.

Table B.9.6-1: In-field exposure calculation for Bulldock 25 EC

Crop	Max. single application rate [g as/ha]	Max. number of Applications	MAFfoliar/MAFsoil	PER (foliar) [g as/ha]	PER (soil) [g as/ha]
Wheat, potato	7.5	2	1.7 / 1.9	12.75	14.25
Wheat, potato	12.5	2	1.7 / 1.9	21.25	23.75

The maximum in-field exposure rates (predicted environmental rate [PER]) to foliar-dwelling organisms are 12.75 g as/ha and 21.75 g as/ha (equivalent to 0.51 L Bulldock 25 EC/ha and 0.85 L Bulldock 25 EC/ha) and to soil-dwelling organisms are 14.25 g as/ha and 23.75 g as/ha (equivalent to 0.57 L Bulldock 25 EC/ha and 0.95 L Bulldock 25 EC/ha), assuming the worst-case of 100 % crop interception and 0 % crop interception, respectively.

Off-field exposure

Exposure of non-target arthropods living in non-target off-field areas to Bulldock EC 25 will mainly be due to spray drift from field applications. Off-field predicted environmental rates (PER-values) were calculated from in-field PERs in conjunction with drift values published by the BBA (2000[2]) as shown in the following equation:
where:

$$Off-field\ PER = \frac{Maximum\ in-field\ PER\ x \left(drift\ percentile / 100 \right)}{vegetation\ distribution\ factor\ (vdf)}$$

vdf = vegetation distribution factor used in combination with test results derived from 2-dimensional exposure set-ups
To account for interception and dilution by three-dimensional vegetation in off-crop areas, a vegetation distribution or dilution factor (vdf, see above) is incorporated into the equation when calculating off-field exposure in conjunction with toxicity endpoints derived from two-dimensional studies (e.g. glass plate or leaf discs). A vdf of 10 is recommended in the ESCORT 2 report when the off-field risk assessment is based on toxicity endpoints obtained in a test design with two-dimensional exposure but has been questioned. Germany considers a vdf of five as a more reliable value to extrapolate from a two dimensional exposure situation to the exposure situation in the field. The exposure estimation was based mainly on the 'Retention Area Index' (RAI) characterising the total retention area of sprayed plant protection products in a canopy per base area. As a 'realistic worst case

Standard Characteristics of Non-Target Arthropod Regulatory Testing. Wageningen, The Netherlands, 46 pp.

[2] BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft) (2000): Abdrifteckwerte für Flächen- und Raumkulturen sowie für den gewerblichen Gemüse-, Zierpflanzen- und Beerenobstanbau. Bundeanzeiger 100, 26. Mai 2000, Köln, pp. 9879.

scenario, meadow canopies < 20 cm height was chosen (Koch and Weisser, 2004[3]; German Federal Environment Agency UBA, 2006[4]).
The derived vdf of 5 agrees well with field data by Koch et al. (2003)[5], who compared measured residues of plant protection products on two dimensional surfaces with the measured residues on meadows next to a treated area (factor of 4.4 to 6.5 between median spray residues on leaves when a standard nozzle was used for spray application). For endpoints resulting from 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the vdf is not used.

Table B.9.6-2: Off-field exposure calculation for Bulldock 25 EC (Tier 1)

Crop / application rate	Study type	In-field foliar PER [g as/ha]	risk mitigation measures	drift factor [% drift/100]	Vegetation distribution factor	Off-field foliar PER [g as/ha]
Wheat, potato / 7.5 g as/ha	2-dimensional	12.75	1m	0.0238	5	0.06
			1m + 50 % drift reduction	0.0119	5	0.03
			1 m + 75 % drift reduction	0.00595	5	0.015
			1 m + 90 % drift reduction	0.00238	5	0.00607
			5 m	0.0047	5	0.011990
			5m + 50 % drift reduction	0.00235	5	0.005993
			5 m + 75 % drift reduction	0.00175	5	0.004463
			5 m + 90 % drift reduction	0.00047	5	0.001199

3 Koch H and Weisser P, 2004. Die Gesamtoberflaeche in Saumstrukturen als potentielle Retentionsflaeche fuer Driftpartikel, Retention Area Index (RAI). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 56, 65-69.
4 German Federal Environment Agency (UBA), 2006. Exposure calculation for arthropods in field border structures - selection of an appropriate 'vegetation distribution factor'. Parma.
5 Koch H, Weisser P and Landfried M, 2003. Effect of drift potential on drift exposure in terrestrial habitats. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 55, 181-188.

Wheat, potato / 12.5 g as/ha	2-dimensional	21.25	1m	0.0238	5	0.101115
			1m + 50 % drift reduction	0.0119	5	0.050575
			1 m + 75 % drift reduction	0.00595	5	0.025288
			1 m + 90 % drift reduction	0.00238	5	0.010115
			5 m	0.0047	5	0.019975
			5m + 50 % drift reduction	0.00235	5	0.009988
			5 m + 75 % drift reduction	0.00175	5	0.004463
			5 m + 90 % drift reduction	0.00047	5	0.001998
Tomato, 17.5 g as/ha greenhouse	Due to the non-volatile property of beta-cyfluthin, an exposure of the area outside the greenhouse is not expected.					

B.9.6.2Risk assessment

B.9.6.2.1 Tier 1 risk assessment for in-field exposure

The risk for non-target arthropods exposed in-field to Bulldock EC 25 was assessed by calculating the hazard quotient (HQ = exposure/toxicity) as the ratio of the predicted environmental rate (PER) and the lowest lethal rate (LR_{50}) estimated in standard toxicity tests with non-target arthropods according to the formula:

$$\text{In field HQ} = \frac{\text{In - field PER}}{LR_{50}}$$

The resulting HQ in-field values for the standard species are presented in the following table.
It is noted that the tier 1 HQ trigger criterion has been calibrated using laboratory LR_{50}s from glass plate studies on the standard tier 1 indicator species. However, according to the ESCORT II report, "for products where effects on reproduction are expected, sub-lethal parameters (e.g. oviposition) should also be evaluated" in tier 1. Effects on reproduction should be assessed if detected in tier 1 studies. Considering the worst-case character of the exposure conditions in standard tier 1 studies it is considered appropriate to set the tier 1 acceptability criterion for reproductive effects for *T. pyri* and *A. rhopalosiphi* at an HQ of 2 in line with the mortality assessment. Therefore, the tier 1 risk assessment uses the ER_{50} for the most sensitive parameter (mortality or reproduction) in HQ calculations.

Table B.9.6-3: HQ values for non-target arthropods (Tier-1) for in-field exposure

Species	**Intended use**	**LR_{50} [g as/ha]**	**Exposure**	**PER [g as/ha]]**	**HQ**
A. rhopalosiphi	Wheat, potato 7.5 g as/ha	0.163	in-field	12.75	78
	Wheat, potato 12.5 g as/ha		in-field	21.25	130

T. pyri	Wheat, potato 7.5 g as/ha	0.0025	in-field	12.75	5100
	Wheat, potato 12.5 g as/ha		in-field	21.25	8500

PER: Predicted environmental rates ; HQ: Hazard quotient

All in-field HQ triggers for Tier 1 are above the trigger value for both indicator species *A.rhopalosiphi* and *T. pyri* indicating an unacceptable risk to non-target arthropods.

B.9.6.2.2 Tier 1 risk assessment for off-field exposure

In order to assess the risk of Bulldock EC 25 to non-target arthropods in off-field areas, the predicted environmental rate in the off-field is compared to the toxicity endpoints according to the following formula:

$$Off-field\ HQ = \frac{Off-field\ PER}{LR_{50}} \times correction\ factor$$

where:

Correction factor (also 'safety factor') = amounts to 10 in conjunction with Tier I data from tests on glass plates; amounts to 5 for Tier II data from extended laboratory tests/field tests. The factor accounts for extrapolation from testing few representative species to the species diversity expected in off-crop areas.

The risk is considered acceptable if the HQ off-field < 2.
It is noted that the tier 1 HQ trigger criterion has been calibrated using laboratory LR_{50} from glass plate studies on the standard tier 1 indicator species. However, according to the ESCORT II report, "for products where effects on reproduction are expected, sub-lethal parameters (e.g. oviposition) should also be evaluated" in tier 1. Effects on reproduction should be assessed if detected in tier 1 studies. Considering the worst-case character of the exposure conditions in standard tier 1 studies it is considered appropriate to set the Tier 1 acceptability criterion for reproductive effects for *T. pyri* and *A. rhopalosiphi* at an HQ of 2 in line with the mortality assessment. Therefore, the tier 1 risk assessment uses the ER_{50} for the most sensitive parameter (mortality or reproduction) in HQ calculations.

The results of the risk assessment are summarised in the following table.

Table B.9.6-4: HQ values for non-target arthropods (Tier-1) for off-field exposure

Species	**Intended use**	**L/ER$_{50}$ [g as/ha]**	**Exposure**	**PER*$_{off-field}$ x correction factor [g/ha]**	**HQ**
A. rhopalosiphi	Wheat, potato 7.5 g as/ha	0.163	off-field	0.01199	0.07
	Wheat, potato 12.5 g as/ha		off-field	0.019975	0.12
T. pyri	Wheat, potato 7.5 g as/ha	0.0025	off-field	0.01199	4.76
	Wheat, potato 12.5 g as/ha		off-field	0.019975	7,99

PER: Predicted environmental rates ; HQ: Hazard quotient; TER: Toxicity to exposure ratio
*PER off –field with risk mitigation: 5 m + 90 % drift reduction (drift factor = 0.00047)

Considering risk mitigation measures (5 m + 90 % drift reduction), the Tier 1 off-field HQ for Aphidius rhopalosiphi is below the trigger of 2.However, the HQ trigger is not met for *Typhlodromus pyri*. This indicates an unacceptable risk to non-target arthropods.

B.9.6.2.3 Higher tier off-field risk assessment

Leaf dwelling arthropods:
Due to the lack of valid extended laboratory tests and semi-field tests for leaf dwelling arthropods, a tier -2 risk assessment cannot be conducted.
Although several valid aged residue studies with *Coccinella septempunctata* reveal a potential for recolonialisation in regard to this species, they are not appropriate to unburden the results of the tier-1 risk assessment. Based on information from all valid laboratory and extended laboratory studies, *T.*pyri, not C*. septempunctata* turned out to be the most sensitive species.

Soil dwelling arthropods:

Studies on two representative species of this group are available; *Aleochara bilineata* and *Poecilus cupreus* (see Table B.9.5-11).

The study with ***Aleochara bilineata*** is classified as not valid (please refer to B.9.5.2.1).

Poecilus cupreus

A laboratory study was conducted with Bulldock EC 25 formulation on the adult rove beetle *Poecilus cupreus* (Heimbach, 1990, KIIIA1 10.5.1/03). The beetles were exposed to a rate of 7.7 g as/ha applied on sand. There were no effects on mortality and slight effects on food consumption two days after application. Further semi-field studies were conducted covering rates from 8.0 g as/ha to 12.5 g as/ha. At the lower application rates no effects on mortality and food consumption was observed, for the highest rate of 12.5 g as/ha an effect on food consumption of 100 % was observed. Based on the highest rate tested, an in-field risk cannot be excluded.
A second laboratory test conducted with Bulldock EC 25 on *Poecilus cupreus* larvae (Neumann, 2001, KIIIA1 10.5.2/02) were submitted with the dossier for the second representative formulation Montur Forte FS 230 (but discribed within this document) revealed an significantly higher sensitivity to larvae (LR50> 0.04 mg as/kg soil; LR100 ≤ 0.4 mg as/kg soil; NOEC < 0.04 mg/as kg/soil). As the maximum $PECsoil_{accu}$ (potatos; BBCH 10, 2 x12.5 g /ha) is 0.0242 mg as/kg soil (dw), the risk has to be further addressed in the context of full fauna field studies.

B.9.6.2.4 Refined in-field risk assessment based on full fauna field studies

Four in-field studies were submitted. The RMS evaluated all studies on the basis of de Jong et al. (2010):

1. Field study in an alfalfa field in Spain (Mack, 2013, R-28693, KIIIA1 10.5.3/05)
The study was conducted in alfalfa. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

Arthropods caught are only out of 7 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis.

The following orders are lacking completely:
1. Dermaptera

2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera

The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

Conclusion: The study is classified as R3 – not reliable.

Field study in a pome fruit orchard in northern Germany (Knäbe, 2013, R-28694, KIIIA110.5.3/06)

The study was conducted in orchard. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis.
The following orders are lacking completely:

1. Lepidoptera
2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera
6. Dermaptera

The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

Conclusion: The study is classified as R3 – not reliable.

Field study in cereals in southern England (Vinall, 2005, R-19598, KIIIA1 10.5.3/03)

The applied test formulation was not the representative product Bulldock EC 25, but the cyfluthrin formulation Baythroid EC 050. The interpretation of study results in terms of the representative use of Bulldock EC25 is associated with a high level of uncertainty.

Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis. In
The following orders are lacking completely:

1. Lepidoptera
2. Neuroptera
3. Orthoptera
4. Psocoptera
5. Thysanoptera
6. Dermaptera

Conclusion: The study is classified as R3 – not reliable.

Field study in an orchard in south-west France (Vinall, 2006, R-19592, KIIIA1 10.5.4/04)

The study was conducted in orchard. The representativeness for areas of intended use (cereals, potatos) remains unclear. No justification is given.

The applied test formulation was not the representative product Bulldock EC 25, but the cyfluthrin formulation Baythroid EC 050. The interpretation of study results in terms of the representative use of Bulldock EC25 is associated with a high level of uncertainty.

Arthropods caught are only out of 8 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis
The following orders are lacking completely:

1. Orthoptera
2. Thysanoptera
3. Dermaptera
4. Collembola

Conclusion: The study is classified as R3 – not reliable.

Conclusions of risk assessment based on full fauna field studies

As all four studies show strong shortcomings, they are classified as R3 - not reliable.
Due to their incomparable study design (different crops, different sprayed products) an overall assessment of all four studies is not possible.
Consequently, no reliable data about the toxicity of Bulldock EC 25 to non-target arthopods in-field areas are available. Accordingly, a higher tier risk assessment cannot be conducted.

B.9.6.2.5 Higher tier off-field risk assessment

Off- field study in a meadow in Germany (Mack, 2014, R-30607, KIIIA1 10.5.3/07)

The submitted off-field study was conducted to assess the impact of drift rates of Bulldock EC 25 on non-target arthropod fauna in a meadow.
The reliability of the study was valued according criterias from de Jong et al. 2010.
The following shortcomings were determined:

1. Arthropods caught are only out of 6 orders. According de Jong et al. (2010) a minimum of 12 orders have to be evaluated in representative agro-ecosystems in Europe. Furthermore a typical field study should have about 50 - 80 taxa available for statistical analysis. In Contrast arthropods sampled in this study belong to only 22 different families (out of 6 orders) . The following orders are lacking completely:
   1. Collembola (above-ground)
   2. Lepidoptera
   3. Neuroptera
   4. Orthoptera
   5. Psocoptera
   6. Thysanoptera

2. The two available valid laboratory studies with the beta-cyfluthrin-formulation Bulldock 25 EC show a high sensitivity of the predacious mite *Typhlodromus pyri*. This sensitivity is also supported by laboratory data of other pyrethroids. Mites as a suborder of *Aranea* (webspiders) were also not sampled. Therefore the taxon known as the most sensitive is lacking, too.

3. The abundance of the sampled (statistically evaluated) taxa few days (-2 and -3 days) before treatment is very low for several species and rises during the study course (especially in the control). Therefore, possible short-term effects right after application (typical for knock-out effects usually caused by pyrethroids) might stay undetectable.

Several reasons for the low general diversity in the plots as well as for the relatively low abundance of sampled taxa before treatment start might be discussed.
Besides changing weather conditions from the first sampling time (average air temperatures: 13-14.6 °C) to the second sampling time (average air temperatures: 21.2 - 29.5 °C), the mowing of the treatment plots 16 days as well as the fertilisation 8 days before treatment are conceivable causes. The latter two are not comprehensible and could have been avoided. Furthermore, agricultural measures are not in line with natural off-crops (like natural meadows).

3. The plot design is very unpropitious. For this reason it cannot be distinguished between recolonisation and real recovery. Only the latter appears to be acceptable for risk assessment of off-crop areas:

1. Plots are regarded as too small (0.09 ha/plot). In contrast de Jong et al. (2010) recommends a minimum plot area of 1 ha.
2. The distance between plots of 10 m is regarded as too close to exclude a recolonisation from less exposed plots and/or control plots to higher treated plots (instead of a real recovery).
3. In general, closed plots (cage) should be favored to exclude recolonisation.

4. Generally, the RMS is of the opinion, that arthropod communities of grassland are not representative for all off-crop areas in Europe. Thus, it's regarded impossible to cover the risk of pesticides to non-target arthropods with one off-field study conducted in one kind of habitat.

Conclusion: The study is classified as R3 – not reliable.

B.9.6.2.6 Overall conclusions on risk to non-target arthropods

In-field
As no valid Tier 2 Studies and no reliable in-field studies are available, the risk assessment remains on tier 1. Based on the endpoints from the laboratory tests with *A. rhopalosiphi* and *T. pyri*, the risk assessment indicates an unacceptable risk to non-target arthropods.

Off-field
As no valid Tier 2 Studies and no reliable in-field studies are available, the risk assessment remains on tier 1.
When considering maximum risk mitigation measures (5 m + 90 % drift reduction), the Tier 1 off-field HQ for Aphidius rhopalosiphi is below the trigger of 2. However, the HQ trigger is not met for *Typhlodromus pyri*. This indicates an unacceptable risk to non-target arthropods.

Due to the non-volatile property of beta-cyfluthin, an exposure of the area outside the greenhouse is not expected. Therefore the risk for non-target arthropods after greenhouse applications in tomatos (17.5 g as/ha, 14 d) is acceptable.

B.9.7 Effects on non-target soil meso- and macrofauna

B.9.7.1 Effects on Earthworms

An acute study on Eisenia fetida with beta-cyfluthrin (technical substance) was submitted and evaluated in the course of the initial Annex I inclusion of beta-Cyfuthrin. This study (Heimbach, 1987) is classified as valid and summarised shortly below.
A new chronic reproduction studies on *Eisenia fetida* with the beta-cyfluthrin representative formulations Bulldock EC 25 was conducted and is summarised in the corresponding below.
For the main soil metabolites FPB-acid and DCVA, two acute studies on *Eisenia fetida* and two reproduction studies are available and summarised below.

Table B.9.7-1: Toxicity of beta-cyfluthrin and metabolites FPB-acid and DCVA to earthworms

Species	Test design	LC50 (mg as/kg soil dw)	NOEC (mg as/kg soil dw)	Reference	reliability
Beta-Cyfluthrin					
Eisenia fetida	14 d acute	>1000 >500 [1]	10 5 [1]	KIIA 8.9.1/01 HBF/RG 83 Heimbach, 1987 M-053564-01-1 R-19143	valid
Bulldock EC 25					
Eisenia fetida	14 d acute	29.7 14.85[1]	1 0.5[1]	KIIIA1 10.6.2 Heimbach, 1988, M-053588-01-2, HBF/RG 85	valid
Eisenia fetida	56 d chronic	4.06 (reproduction) 2.03 (reproduction)	1.65 (reproduction) **0.83** **(reproduction)** [1]	KIIIA1 10.6.3 74484022 Pavic, 2013 M-461275-01-1 R-30148	valid
FPB-acid					
Eisenia fetida	14 d acute	> 63 > 31.5 [1]	1 < 63 <31.5	KIIA 8.9.1/02 09P11RA Moser and Scheffczyk, 2009 M-354192-01-1 R-27979	valid
Eisenia fetida	56 d chronic	-	5.2 (reproduction) **2.6** **(reproduction)** [1]	KIIA 8.9.2/01 kra/Rg-R-143/13 Kratz, 2013a M-468873-01-1 R-34697	valid
DCVA					
Eisenia fetida	14 d acute	122.7 61.35 [1]	< 63 <31.5 1	KIIA 8.9.1/03 09P10RA Moser, 2009 M-356435-01-1 R-27978	valid

Eisenia fetida	56 d chronic	184.76 92.38 1	5.2 (reproduction) **2.6** **(reproduction) [1]**	KIIA 8.9.2/02 kra/Rg-R-157/13 Kratz, 2013b M-468552-01-1 R-34696	valid

Studies shaded in grey have been reviewed as part of the 2002 EU evaluation.
Values in bold: Endpoints used for risk assessment
[1] endpoint corrected/divided with a factor of 2, due to log Pow >2 and peat content of 10 % in study
log Pow results FPB-acid at 23 °C: Log Pow = 2.6 at pH 5, Log Pow = 0.8 at pH 7, Log Pow = -0.5 at pH 9
log Pow results DCVA at 25 °C: Log Pow = 2.5 at pH 5, Log Pow = 0.8 at pH 7, Log Pow = -0.8 at pH 9

KIIIA1 10.6.2

Author:	Heimbach. F.
Title:	Acute toxicity of FCR 4545 EC 025 to earthworms
Date:	19.01.1988
Doc ID:	M-053588-01-2
Report no.:	HBF/RG 85
Guidelines:	OECD Guideline No. 207
GLP:	yes
Validity:	valid

Materials/Study Design:
In accordance with GLP-regulations, FCR 4545 EC 025 (Fl.-No.: 021 according to 004) was tested for acute toxicity to earthworms according to the OECD-Guideline No. 207 (OECD Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests, 4 April 1984).
The test animals were exposed to different concentrations of FCR 4545 EC 025 in an artificial soil consisting of sand, clay mineral and peat. The test compound was thoroughly mixed into the artificial soil. After 14 days, the number of surviving animalsand their weight alteration during the test period was determined.The values given represent nominal concentrations.
Results:
The LC (test duration: 14 days, test species: Eisenia foetida) is 29.7 mg as/kg dry weight substrate (95 %-Confidence limits 26.5 - 33.2 mg/kg).
The 'no-observed-effect-concentration1 (NOEC) is 1 mg as/kg dry, weight substrate, the lowest tested concentration with mortality ('lowest lethal concentration1, LLC) is 18 mg as/kg dry weight of substrate.
Conclusion:
The study was conducted in a soil with 10 % peat.
Thus, endpoints have to be devided by 2.

NOEC = 0.5 mg as/kg soil (dw)
LC_{50} = 14.85 mg as/kg soil (dw)

KIIIA1 10.6.3 (newly submitted with the dossier)

Author:	Pavic, B.
Title:	Effects of Bulldock 25 EC on Reproduction and Growth of Earthworms Eisenia fetida in Artificial Soil with 10 %Peat
Date:	03.07.2013
Doc ID:	M-461275-01-1
Report no.:	74484022
Edition no.:	R-30148
Guidelines:	OECD Guideline No. 222, 2004 and ISO 11268-2, 1998
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed in a 56 day test to six test concentrations of Bulldock 25 EC in artificial soil containing 10 % sphagnum peat and observed for mortality, weight change, feeding activity and reproduction. A negative control group was maintained concurrently. Four replicate test chambers for the test item and eight replicate test chambers for the control were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 18.5, 33.3, 60.0, 108.0, 194.4 and 350.0 mg test item/kg dry soil. Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
A slight mortality of 2.5 % was found at the concentration of 194.4 mg Bulldock 25 EC /kg soil, which
was not statistically significantly different compared to the control, where no mortality was observed. At the highest test concentration of 350.0 mg test item/kg soil a mortality of 97.5 % was observed, which was significantly increased compared to the control(Fisher's Exact Test, α = 0.05 one-sided greater).
The body weight changes of the earthworms after 4 weeks of exposure to Bulldock 25 EC were not statistically significantly different compared to the control up to and including the test concentration of 194.4 mg test item/kg soil.
The reproduction rates were not significantly different compared to the control up to and including the test concentration of 60.0 mg test item/kg soil. At the concentrations of 108.0 mg test item/kg soil and above the numbers of juveniles were significantly reduced compared to the control (Williams t-test, α = 0.05, one-sided smaller).
one-sided smaller).
At the test concentration of 194.4 mg Bulldock 25 EC/kg soil some of the worms did only burrow into soil after 3 days. At the test concentration of 350.0 mg Bulldock 25 EC/kg soil some of the worms did only burrow into the soil within 14 days after application. At the lower concentrations no worm was observed on the soil surface 15 min after introduction. The feeding activity was reduced at the concentration of 350.0 mg Bulldock 25 EC/kg soil whereas the food intake in the remaining treatment groups was comparable to the control.

The No Observed Effect Concentration (NOEC) for mortality, growth, and feeding activity of the earthworm *Eisenia fetida* was determined to be 194.4 mg Bulldock 25 EC/kg soil. The LC50 was determined to be 260.8 mg Bulldock 25 EC/kg soil (95 % confidence limits: 237.9 to 286.0 mg test item/kg soil). This is equivalent to 7.18 mg as/kg soil.
The No Observed Effect Concentration (NOEC) for reproduction was determined to be the concentration of 60.0 mg Bulldock 25 EC/kg soil (equivalent to 1.65 mg as/kg soil). The EC50 was determined to be 147.7 mg Bulldock 25 EC/kg soil (equivalent to 4.06 mg as/kg soil) (95 % confidence limits were not determinable).
As the peat content of the used artificial soil was 10 % and the log Pow of the active substance is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the NOECreproduction is 0.825 mg as/kg soil, EC_{50} reproduction is 2.03 mg as/kg soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Bulldock 25 EC
Description:	Emulsifiable concentrate, liquid
Lot/Batch#:	92110164
Purity	2.752 % (w/w) (analysed)

2. Vehicle and/or positive control:	Carbendazim
3. Test organisms:	
Species:	Earthworm (*Eisenia fetida*)
Age:	adults with clitellum
Weight:	301-600 mg
Source:	In house bred
Diet/Food:	ground cattle manure was used as food
Acclimatisation:	1 day, in artificial soil, under test conditions
4. Environmental conditions:	
Temperature:	18-22 °C
Photoperiod:	16 h light (400 – 800 lux): 8 hours dark
pH:	5.9 – 6.0 (start) 6.2 – 6.4 (end)
Water content:	25.7 – 28.0 % (51.4 - 56.0 % of the maximum WHC) (start) 26.8 - 29.5 % (53.6 - 59.0 % of the maximum WHC) (end)
Composition of artificial soil:	10 % sphagnum peat 20 % kaolin clay calcium carbonate added to adjust pH to 6.0 ± 0.5 approx. 70 % quartz sand Deionised water

B. STUDY DESIGN

1. Experimental treatments
Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 222, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 70 % industrial quartz sand and calcium carbonate). Four replicate test chambers were maintained in each treatment and eight replicate test chambers were maintained for the control, with 10 worms in each test chamber. Nominal test concentrations of 18.5, 33.3, 60.0, 108.0, 194.4 and 350.0 mg test item/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to 50 ±10 % dry weight using deionised water. Negative control soil was treated with deionised water only.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.
Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations
Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). Mortality, weight change, feeding activity and reproduction rate were determined.

3. Statistical calculations
Mortality was analysed for significance by using the Fisher's Exact Test (one-sided greater, $\alpha = 0.05$). The EC and LC values and their 95 % confidence limits were calculated by applying Probit-Analysis (Finney, 1971).
The body weight change and reproduction data were tested for normal distribution ($\alpha = 0.05$) using the Shapiro-Wilk´s test (for body weight change) and Kolmogorov-Smirnov test (for reproduction) homogeneity of variance ($\alpha = 0.05$) using the Levene's test (for body weight change and reproduction).

As the data for body weight changes and reproduction were normally distributed and homogeneous, Williams t-test was used to compare treatment and control values (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$).
The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was seen in the four lower test concentrations. A slight mortality of 2.5 % was found at the concentration of 194.4 mg Bulldock 25 EC/kg soil, which was not statistically significantly different compared to the control, where no mortality was observed. At the highest test concentration of 350.0 mg Bulldock 25 EC/kg soil a mortality of 97.5 % was observed, which was significantly increased compared to control(Fisher's Exact Test, $\alpha = 0.05$, one -sided greater). This indicates a steep dose/response between the two highest concentrations.
The NOEC for mortality was determined to be 194.4 mg Bulldock 25 EC/kg soil. The LC10 was determined to be 215.2 mg Bulldock 25 EC/kg soil (95 % confidence intervals of 188.9 to 236.1 mg Bulldock 25 EC/kg soil), the LC20 was determined to be 229.9 mg Bulldock 25 EC/kg soil (95 % confidence intervals of 205.3 to 251.2 mg Bulldock 25 EC/kg soil) and the LC50 was determined to be 260.8 mg Bulldock 25 EC/kg soil (95 % confidence intervals of 237.9 to 286.0 mg Bulldock 25 EC/kg soil, Probit Analysis).
The body weight changes were not statistically significantly different compared to the control up to and including the test concentration of 194.4 mg Bulldock 25 EC/kg soil. At the highest test concentration of 350.0 mg Bulldock 25 EC/kg soil the body weight was significantly reduced compared to the control (Williams t-test, two-sided). However, the result is based on one surviving worm only.
The NOEC for body weight changes was determined to be 194.4 mg Bulldock 25 EC/kg soil.
The reproduction rates were not significantly different compared to the control up to and including the test concentration of 60.0 mg Bulldock 25 EC/kg soil. At the concentrations of 108.0 mg Bulldock 25 EC/kg soil and above the numbers of juveniles were significantly reduced compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller). The NOEC for reproduction was determined to be 60.0 mg Bulldock 25 EC/kg soil.
The EC10 was determined to be 45.2 mg Bulldock 25 EC/kg soil (95 % confidence intervals not determinable), the EC20 was determined to be 67.8 mg Bulldock 25 EC/kg soil (95 % confidence intervals not determinable) and the EC50 was determined to be 147.7 mg Bulldock 25 EC/kg soil (95 % confidence intervals not determinable, Probit Analysis).

Table B.9.7-2: Effect of Bulldock 25 EC on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Bulldock 25 EC [mg/kg soil dry weight]	**Control**	**18.5**	**33.3**	**60.0**	**108.0**	**194.4**	**350.0**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	2.5	97.5
Significance 1)	-	n.s	n.s	n.s	n.s	n.s	*
Weight change (day 28) [%]	45.6	54.1	52.1	46.0	52.8	50.9	-9.44)
Significance 2)	-	n.s	n.s	n.s	n.s	n.s	*
Mean No. of juveniles (day 56)	188	166	171	163	102	111	0
Significance 2)	-	n.s	n.s	n.s	*	*	*
Reproduction in [%] of control (day 56)	-	88.5	90.8	86.5	54.2	59.2	0.0
Food consumption [g]	20.0	20.0	19.5	19.3	22.3	20.8	6.3
Endpoints [mg Bulldock 25 EC/kg soil]							
NOEC (day 28 mortality and weight)	**194.4**						
LC values (mortality)[3)]	LC_{10}		LC_{20}		LC_{50}		
	215.5		229.9		260.8		

NOEC (day 56 reproduction)	**60.0**		
EC values (reproduction)[3)]	LC_{10}	LC_{20}	LC_{50}
	45.2	67.8	147.7

- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
1)Fisher's Exact Test, α =0.05, one -sided greater
2) Williams t-test, α =0.05, two-sided for weight changes and one-sided smaller for reproduction
3) Probit analysis
4) Value is based only on one worm

With a control mortality of 0 %, a number of juvenile worms per replicate of 158 to 239 and a coefficient of variance of the reproduction of 16.0 %, all validity criteria according to guideline OECD 222 are therefore fulfilled.
In the test with the reference item Luxan Carbendazim 500 FC performed two months before the present study (IBACON Study Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher; the EC50 for reproduction was calculated as 1.7 mg carbendazim/kg soil. These results show the sensitivity of the test animals.

III. CONCLUSIONS

The No Observed Effect Concentration (NOEC) for mortality, growth, and feeding activity of the earthworm Eisenia fetida was determined to be 194.4 mg Bulldock 25 EC/kg soil. The LC50 was determined to be 260.8 mg Bulldock 25 EC/kg soil (95 % confidence limits: 237.9 to 286.0 mg test item/kg soil). This is equivalent to 7.18 mg as/kg soil.
The No Observed Effect Concentration (NOEC) for reproduction was determined to be the concentration of 60.0 mg Bulldock 25 EC/kg soil (equivalent to 1.65 mg as/kg soil). The EC50 was determined to be 147.7 mg Bulldock 25 EC/kg soil (equivalent to 4.06 mg as/kg soil) (95 % confidence limits were not determinable).
As the peat content of the used artificial soil was 10 % and the log Pow of the active substance is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the NOECreproduction is 0.825 mg as/kg soil, EC_{50} reproduction is 2.03 mg as/kg soil.

B.9.7.2Effects on non-target soil meso- and macrofauna (other than earthworms)

Toxicity

New studies have been conducted exposing Hypoaspis aculeifer and Folsomia candida to beta-cyfluthrin. In addition, studies on Hypoaspis aculeifer and Folsomia candida with the metabolites FPB-acid and DCVA (permethric-acid) are available and summarised below.
For *Folsomia candida* a study is available with the representative formulation Bulldock 25 EC and is presented below.

All studies summarised below were conducted in artificial soil with a peat content of 5 % or in LUFA 2.1. soil with an assumed peat content of < 5 %.
For this reason, the resulting endpoints for beta-cyfluthrin (logpow = 5.9) and its metabolites FPB-acid (log Pow = 2.6) and DCVA (log Pow = 2.5) are not divided by 2.
Rational:
RMS acknowledges the decision by EFSA during the peer review 91 of penflufen from April 2012 regarding the division of endpoints for lipophilic substances conducted with 5 % organic matter (peat) in the test soil. However, RMS would not support the decision to divide the endpoint of the soil macro-organisms studies performed in a standard soil with an OM content of 5 % by 2. The approach of dividing endpoints is published in the old Guidance document (GD) for terrestrial ecotoxicology

(SANCO/10329/2002). By the time the old GD was discussed, tests for soil organisms were conducted with testing soils containing 10 % peat only. Therefore, a division of endpoints was necessary to address the bioavailability of chemicals for soil organisms in test soils. Assuming a linear correlation between the OM-content and the bioavailability of chemicals for test organisms, it would be inconsistent to divide endpoints by 2 from tests conducted with soils containing 5 % OM.
A linear correlation between OM-content and bioavailability for soil organisms provided, tests conducted with soils containing 10 % OM had to be divided by 4 if tests conducted with soils containing 5 % OM are divided by two.
Moreover, if the division of endpoints by two is used for both OM-contents (5 % + 10 %), there would not be an incentive for applicants to submit new studies on soil organisms with soils containing 5 % OM anymore.

This question should urgently be clarified by an eligible committee on EU-level, independentlyfrom the evaluation of active substances according to regulation EC No 1107/2009.
Unless the further approach how to handle tests with different OM-content in testing soils is not clear, tests on soil organisms with 5 % OM in testing soils should not be divided by two.

Table B.9.7-3: Toxicity of beta-cyfluthrin, its relevant metabolites FPB-acid, DCVA and Bulldock EC 25 to soil meso- and macroorganisms (other than earthworms

Species	Test design	NOEC (reproduction) (mg as/kg dry soil)	Reference	reliability
Beta-Cyfluthrin				
Hypoaspis aculeifer	14 d chronic	**0.97**	KIIA 8.9.2/03 74501089 Pavic, 2012 M-476271-01-1; R-30149	valid
Folsomia candida	28 d chronic	**56**	KIIA 8.9.2/04 FRM-Coll-172/14 Frommholz, 2014 M-475305-01-1; R-34698	valid
Bulldock 25 EC				
Folsomia candida	28 d chronic	**1.592**	KIII1 10.6.6/01 IRV-13-7 McCormac, 2014 R-33352	valid
FPB-acid				
Hypoaspis aculeifer	14 d chronic	**297**	KIIA 8.9.2/05 P14HR Moser and Scheffczyk, 2005a M-258697-01-1; R-23564	valid
Folsomia candida	28 d chronic	**28**	KIIA 8.9.2/06 FRM-Coll-144/12 Frommholz, 2012a M-440962-01-1; R-34695	valid

DCVA				
Hypoaspis aculeifer	14 d chronic	**≥ 316 100 (mortality)**	KIIA 8.9.2/07 P15HR Moser and Scheffczyk, 2005b M-259607-01-1; R-23565	valid
Folsomia candida	28 d chronic	**18**	KIIA 8.9.2/08 FRM-Coll-143/12 Frommholz, 2012b M-440379-01-1; R-34694	valid

Values in bold: Endpoints used for risk assessment

A study addressing the toxicity of Bulldock EC 25 to *Hypoaspis aculeifer* is not available. Therefore, the risk assessment in chapter B.9.8 is based on data of theactive substance.
However, comparing the endpoints (NOEC) of beta-cyfluthrin and the Bulldock EC 25 for *Folsomia candida*, a 35 fold higher toxicity of Bulldock EC 25 can be determined.
As TER values for *Hypoaspis aculeifer* divided by 35 (assuming a comparable difference in toxicity) would be below the acceptablity criterion of 5, the toxicity and, therefore, the risk of Bulldock EC 25 to *Hypoaspis aculeifer* can not be sufficiently assessed on the basis of results fort he active substance.

Consequently, a chronic *Hypoaspis aculeifer* study with Bulldock EC 25 is needed.
Thus, a data gap is defined.

KIII1 10.6.6/01 (newly submitted with the dossier)

Author:	McCormac,A.
Title:	Bulldock 25 EC – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae)
Date:	31.03.2014
Doc ID:	
Report no.:	R-33352
Edition no.:	IRV-13-7
Guidelines:	OECD 232(2009)
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, ten collembolans (11 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed in a 28 day test to eight concentrations of Bulldock 25 EC. The treatment rates were 1.63, 2.94, 5.3, 9.53, 17.1, 30.9, 55.6 and 100 mg formulation/kg soil dry weight. These variants were compared to a control treatment of purified water and a toxic reference treatment.
Mortality and reproduction were determined after 28 days. The adult and juvenile collembola of each vessel were counted. All validity criteria according to the guidelines were fulfilled.
In a laboratory test with Bulldock 25 EC and the springtail *Folsomia candida*, the 28-day LC_{50} was found to be > 100 mg product/kg soil dry weight (i.e. > 2.863 mg as/kg soil dry weight, measured content).
Based on statistical analysis of the mortality data, the NOEC was 100 mg product/kg soil dry weight.
In terms of springtail reproduction, the EC_{50} for Bulldock 25 EC was estimated to be 142.6 mg product/kg soil dry weight (i.e. 4.083 mg as/kg soil dry weight, measured content). The EC_{20} was 13.4 mg product/kg soil dry weight and the EC_{10} was 6.1 mg product/kg soil dry weight. Based on

statistical comparison with the control, the 28-day NOEC for Bulldock 25 EC was 55.6 mg product/kg soil dry weight (i.e. 1.592 mg as/kg soil dry weight, measured content).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC
Active ingredient:	beta-Cyfluthrin
Description:	colourless, liquid
Lot/Batch#:	92110454
Content:	Analysed: 2.58 % w/v (25.8 g/L)

2. Vehicle and/or positive control:	Betosip 114 (nominally 114 g/L phenmedipham), 200 mg product/kg soil dry weight

3. Test organisms:

Species:	*Folsomia candida* (Collembola, Isotomidae)
Age:	11 days old
Source:	Cultured in-house at Test Facility (original source: Syngenta Ltd., Jealott´s Hill International Research Centre, Bracknell, UK)
Diet/Food:	Approximately 30 mg dried granulated baker´s yeast provided 2-3 times per week.

4. Environmental conditions:

Temperature:	20.1- 21.8 °C
Composition of artificial soil:	5 % Sphagnum-peat 20 % Kaolin clay 0.18 % w/w (first range-finding bioassay); 0.20 % w/w (second range-finding and definitive bioassays) calcium carbonate (CaCO3) for the adjustment to pH 6.0±0.5 approximately 74.8 % fine quartz-sand
Soil water content:	50 % of the soil´s maximum water-holding capacity (WHC)
pH:	Test start: 5.96 – 5.99 (1st test run), 5.63 – 6.06 (2nd test run) Test end: 5.81 – 5.86 (1st test run), 5.98 – 6.03 (2nd test run)
Light intensity:	580 -640 Lux
Light cycle:	12 h light : 12 h dark

B. STUDY DESIGN

1. Experimental treatments

Bulldock 25 EC was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at eight application rates, e.g. 1.63, 2.94, 5.3, 9.53, 17.1, 30.9, 55.6 and 100 mg /kg dry soil. In addition a blank control with deionised water and a toxic reference were tested.
Each test item concentration was tested with 40 collembola (4 replicates and 10 collembola per test unit), 80 collembola in the control (8 replicates and 10 collembola per test unit) and 50 collembola in the toxic reference (5 replicates and 10 collembola per test unit).

The collembola were put in glass vessels (volume: 125 mL; diameter: 4.5 cm), covered with plastic lids, filled with artificial soil with the requested test item concentrations and closed.

2. Observations
Light intensities were measured at the start of each bioassay. The temperature and relative humidity (RH) were recorded at hourly intervals and pH were determined at test start and after 28 days. Numbers of surviving adult and living juveniles were counted 28 days after application. Mortality and reproduction were determined after 28 days.

3. Statistical calculations
The 28-day mortality data for the individual test-item treatments were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$). It was the intention that values for the lowestobserved-effect concentration (LOEC) and the no-observed-effect concentration (NOEC) for mortality would be derived from the results of the analysis of the definitive test. Probit regression analysis to calculate the median lethal concentration (LC_{50}) could not be performed, since none of the test-item treatments resulted in > 50 % mortality.
Probit regression analysis was performed on the data for the numbers of progeny in the test-item treatments, in an attempt to derive key effect concentrations, i.e. EC_{50}, EC_{20} and EC_{10} that would be expected to reduce the numbers of F1 progeny by 50 %, 20 % and 10 %, relative to the control. To confirm the data from the test-item treatments were suitable for analysis of variance (ANOVA), they were first subjected to the Shapiro-Wilk test for normality ($\alpha = 0.05$), and Levene's test for homogeneity ($\alpha = 0.05$). The numbers of F1 progeny produced in the individual test-item treatments were then compared to numbers in the control, using one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). The LOEC and the NOEC for reproduction were determined from the results.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50}, EC_{50} and NOEC values are given below based on nominal concentrations.

Endpoints	Bulldock 25 EC	
	[mg product/kg dry soil]	[mg as/kg dry soil]
NOEC (mortality)	100.0	2.863
LC_{50} (mortality)	> 100.0	> 2.863
NOEC (reproduction)	55.6	1.592
EC_{50} (reproduction)	142.6	4.083
EC_{20} (reproduction)	13.4	0.384
EC_{10} (reproduction)	6.1	0.175

B. OBSERVATIONS

Table B.9.7-4: The effect of Bulldock 25 EC on the mortality of springtails after 28 days

Bulldock 25 EC [mg/kg soil dry weight]	Adult mortality[a] (%)	Corrected mortality[b] (%)
Control	13	-
1.63	8	0
2.94	10	0
5.3	5	0
9.53	15	3
17.1	15	3
30.9	20	9
55.6	10	0
100	25	14
toxic reference	48*	41

a) The results were compared using Fisher's Exact Test ($\alpha = 0.05$). Values that differed significantly from the control

are marked with an asterisk (*).
b) Derived using Abbott's formula.

Table B.9.7-5: The effect of Bulldock 25 EC on the reproduction of springtails after 28 days

Bulldock 25 EC [mg/kg soil dry weight]	Mean no. progeny per replicate[a]	% change relative to the control[b]
Control	609	-
1.63	614	-0.8
2.94	596	2.2
5.3	584	4.1
9.53	513	15.8
17.1	487	20.0
30.9	434	28.7
55.6	473	22.4
100	225*	63.0
toxic reference	47*	92.3

a) Test-item treatments were compared to the control by one-way ANOVA and Dunnett's t-test (α = 0.05). The toxic reference treatment was compared to the control by t-test for unmatched pairs (α = 0.05). Means marked with an asterisk (*) differed significantly from the control.
b) A positive value indicates a decrease in reproduction, and a negative value an increase, relative to the control

Validity
All validity criteria for the study were met, as adult mortality in the control treatments did not exceed 20 % (actual value 13 %), the mean number of juveniles per test unit was > 100 in the control (actual 609) at test end and the coefficient of variation (CoV) of the control reproduction was <30 % (actual 12.5 %).

III. CONCLUSIONS

In a laboratory test with Bulldock 25 EC and the springtail *Folsomia candida*, the 28-day LC_{50} was found to be > 100 mg product/kg soil dry weight (i.e. > 2.863 mg as/kg soil dry weight, measured content). Based on statistical analysis of the mortality data, the NOEC was 100 mg product/kg soil dry weight.
In terms of springtail reproduction, the EC_{50} for Bulldock 25 EC was estimated to be 142.6 mg product/kg soil dry weight (i.e. 4.083 mg as/kg soil dry weight, measured content). The EC_{20} was 13.4 mg product/kg soil dry weight and the EC10 was 6.1 mg product/kg soil dry weight. Based on statistical comparison with the control, the 28-day NOEC for Bulldock EC 25 was 55.6 mg product/kg soil dry weight (i.e. 1.592 mg as/kg soil dry weight, measured content).

B.9.8 Risk assessment for non-target soil meso- and macrofauna

The maximum PEC_{soil} values were calculated following the recommendations of the FOCUS soil working group (FOCUS, 1997) assuming a soil depth of 5 cm, a bulk density of 1.5 g/cm3 and application rates. For details please refer to Volume3_CP_ Bulldock EC 25_B8.

The chronic risk for earthworms, other non-target soil macro- and mesofauna and organic matter breakdown resulting from an exposure to Bulldock EC 25 / beta-cyfluthrin as well as the major soil degradation products of beta-cyfluthrin was assessed by comparing the maximum PEC_{SOIL} with the NOEC value to generate chronic TER values. The TER_{LT} was calculated as follows:

$$TER_{LT} = \frac{NOEC\,(mg/kg)}{PEC_{soil}\,(mg/kg)}$$

The results of the risk assessment are summarised in the following table.

Table B.9.8-1: TER values for earthworms and other soil macro- and mesofauna (Tier-1), wheat, 2x7.5 g as/ha, interception: 25 %, 14 d

Species	Test item	Time scale	Endpoint [mg/kg as soil dw]	Max. PEC_{SOIL} [mg/kg soil dw]	TER
Eisenia fetida	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	0.83	0.0128	64.84
	FPB-acid	Chronic	2.6	0.0009	2888.89
	DCVA	Chronic	2.6	0.0025	1040.00
Folsomia candida	beta-Cyfluthrin	Chronic	56	0.0128	4375.00
	FPB-acid	Chronic	28	0.0009	31111.11
	DCVA	Chronic	18	0.0025	7200.00
	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	1.592	0.0128	124.38
Hypoaspis aculeifer	beta-Cyfluthrin	Chronic	0.97	0.0128	75.78
	FPB-acid	Chronic	297	0.0009	330000.00
	DCVA	Chronic	100	0.0025	40000.00

Table B.9.8-2: TER values for earthworms and other soil macro- and mesofauna (Tier-1). wheat. 2x12.5 g as/ha. interception: 25 %. 14 d

Species	Test item	Time scale	Endpoint [mg/kg soil dw]	Max. PEC_{SOIL} [mg/kg soil dw]	TER
Eisenia fetida	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	0.83	0.0213	38.97
	FPB-acid	Chronic	2.6	0.0014	1857.14
	DCVA	Chronic	2.6	0.0042	619.05
Folsomia candida	beta-Cyfluthrin	Chronic	56	0.0213	2629.11
	FPB-acid	Chronic	28	0.0014	20000.00
	DCVA	Chronic	18	0.0042	4285.71
	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	1.592	0.0213	74.74
Hypoaspis aculeifer	beta-Cyfluthrin	Chronic	0.97	0.0213	45.54
	FPB-acid	Chronic	297	0.0014	212142.86
	DCVA	Chronic	100	0.0042	23809.52

Table B.9.8-3: TER values for earthworms and other soil macro- and mesofauna (Tier-1): potato. 2x7.5 g as/ha. interception: 15 %. 14 d

Species	Test item	Time scale	Endpoint [mg/kg soil dw]	Max. PEC_{SOIL} [mg/kg soil dw]	TER
Eisenia fetida	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	0.83	0.0143	58.04
	FPB-acid	Chronic	2.6	0.001	2600.00

	DCVA	Chronic	2.6	0.0028	928.57
Folsomia candida	beta-Cyfluthrin	Chronic	56	0.0143	3916.08
	FPB-acid	Chronic	28	0.001	28000.00
	DCVA	Chronic	18	0.0028	6428.57
	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	1.592	0.0143	111.33
Hypoaspis aculeifer	beta-Cyfluthrin	Chronic	0.97	0.0143	67.83
	FPB-acid	Chronic	297	0.001	297000.00
	DCVA	Chronic	100	0.0028	35714.29

Table B.9.8-4: TER values for earthworms and other soil macro- and mesofauna (Tier-1). Potato, 2x12.5 g as/ha interception: 15 %. 14 d

Species	**Test item**	**Time scale**	**Endpoint [mg/kg soil dw]**	**Max. PEC_{SOIL} [mg/kg soil dw]**	**TER**
Eisenia fetida	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	0.83	0.0242	34.30
	FPB-acid	Chronic	2.6	0.0016	1625
	DCVA	Chronic	2.6	0.0047	553.19
Folsomia candida	beta-Cyfluthrin	Chronic	56	0.0242	2314.05
	FPB-acid	Chronic	28	0.0016	17500
	DCVA	Chronic	18	0.0047	3829.79
	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	1.592	0.0242	65.79
Hypoaspis aculeifer	beta-Cyfluthrin	Chronic	0.97	0.0242	40.08
	FPB-acid	Chronic	297	0.0016	185625
	DCVA	Chronic	100	0.0047	21276.60

Table B.9.8-5: TER values for earthworms and other soil macro- and mesofauna (Tier-1): tomatoes greenhouse. 2 x 17.5 g as/ha. interception: 50 %. 14 d)

Species	**Test item**	**Time scale**	**Endpoint [mg/kg soil dw]**	**Max. PEC_{SOIL} [mg/kg soil dw]**	**TER**
Eisenia fetida	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	0.83	0.0152	54.61
	FPB-acid	Chronic	2.6	0.0010	2600.00
	DCVA	Chronic	2.6	0.0030	866.67
Folsomia candida	beta-Cyfluthrin	Chronic	56	0.0152	3684.21
	FPB-acid	Chronic	28	0.0010	28000.00
	DCVA	Chronic	18	0.0030	6000.00
	beta-Cyfluthrin (Bulldock 25 EC)	Chronic	1.592	0.0152	104.74
Hypoaspis	beta-Cyfluthrin	Chronic	0.97	0.0152	63.82

aculeifer	FPB-acid	Chronic	297	0.0010	297000.00
	DCVA	Chronic	100	0.0030	33333.33

The TER_{LT} values exceed the relevant decision-making criteria of 5 for earthworms and other soil macro- and mesofauna. Therefore. it can be concluded that chronic risk to earthworms and other soil macro- and mesofauna for beta-cyfluthrin from the use of Bulldock 25 EC in all crops according to the proposed good agricultural practice will be acceptable.

A study addressing the toxicity of Bulldock EC 25 to *Hypoaspis aculeifer* is not available. Therefore, the risk assessment is based on data of theactive substance.
However, comparing the endpoints (NOEC) of beta-cyfluthrin and the Bulldock EC 25 for *Folsomia candida*, a 35 fold higher toxicity of Bulldock EC 25 can be determined.
As TER values for *Hypoaspis aculeifer* divided by 35 (assuming a comparable difference in toxicity) would be below the acceptablity criterion of 5, the toxicity and, therefore, the risk of Bulldock EC 25 to *Hypoaspis aculeifer* can not be sufficiently assessed on the basis of results fort he active substance.

Consequently, a chronic *Hypoaspis aculeifer* study with Bulldock EC 25 is needed.
Thus, a data gap is defined.

B.9.9 Effects on soil nitrogen transformation

Toxicity

There are three studies available of which two were conducted with beta-cyfluthrin and one with the formulation Bulldock 25 EC. The studies on the active ingredient have been reviewed for Annex I inclusion of beta-cyfluthrin. Additionally. two nitrogen mineralisation studies with the two major soil metabolites (i.e. FPB-acid and DCVA) were conducted and are summarised in Table B.9.9-1.

Table B.9.9-1: Effects on soil micro-organisms

Test design	NOEC (reproduction) (mg as/kg dry soil)	Reference	reliability
Beta-Cyfluthrin			
Nitrogenmineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation by day 28 at 0.018 and **0.18 kg/ha**	KIIA 8.10.1/02 BSI/47987 Blumenstock. 1987 M-054489-01-2 R-19148	valid
Carbonmineralisation 28-day study	No significant effects (>25 %) on microbial respiration by day 28 at 0.018 and **0.18 kg/ha**	KIIA 8.10.1/01 AJO/46887 Anderson. 1987 M-054544-01-2 R-19147	valid
FPB-acid			
Nitrogenmineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation at 0.012 mg/kg dry soil and 0.125 mg/kg dry soil. corresponding to 0.009 kg and 0.094 kg test item/ha. respectively	KIIA 8.10.1/03 13 10 48 016 N Schulz. 2013a M-454537-01-1 R-34704	valid
DCVA			
Nitrogen - mineralisation	No significant effects (>25 %) on	KIIA 8.10.1/04 13 10 48 017 N	valid

28-day study	nitrogen mineralisation at 0.011 mg/kg dry soil and 0.112 mg/kg dry soil. corresponding to 0.008 kg and 0.084 kg test item/ha. respectively	Schulz. 2013b M-454538-01-1 R-34705	
Beta-Cyfluthrin 25 EC			
Nitrogen - mineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation at 0.96 mg/kg dry soil and 9.61 mg/kg dry soil. corresponding to 0.8 L and 8.0 L test item/ha. respectively	KIIIA1 10.7.1 10 48 084 N Schulz. 2011 R-28684	valid

KIIIA1 10.7.1 (newly submitted with the dossier)

Author:	Schulz. L.
Title:	Bulldock 25 EC – Effects on the activity of soil microflora (Nitrogen test)
Date:	20.10.2011
Doc ID:	
Report no.:	11 10 48 084 N
Edition no.:	R-28684
Guidelines:	OECD Guideline 216 (2000)
GLP:	yes
Validity:	valid

Executive Summary

Nitrogen transformation (NO_3-nitrogen production) of test item treated soil was compared with a non-treated control soil. Three replicates were applied for the control and both test item treatments, namely 0.96 mg test item/kg dry soil (corresponding to an application rate of 0.8 L test item/ha) and 9.61 mg test item/kg dry soil (corresponding to an application rate of 8 L test item/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm_3. Soil samples were incubated at 19.5-21.1 °C. while stored in test vessels in the dark.NH_4-nitrogen. NO_3- and NO_2-nitrogen were determined 0. 7. 14 and 28 days after treatment.
No adverse effects of the test item on nitrogen transformation in soil were observed at both tested concentrations after 28 days.

Therefore it is concluded that Bulldock 25 EC has no significant long term effect on nitrogen transformation in soil at concentrations of 0.96 mg/kg dry soil and 9.61 mg/kg dry soil corresponding to 0.8 L and 8.0 L test item/ha, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Bulldock 25 EC

Description: Emulsifiable concentrate (EC). appearance: yellow to brown liquid

Lot/Batch#:	0111311
Purity	active ingredient: beta-cyfluthrin (2.5 % w/w (nominal); 2.7 % w/w (analysed))
2. Reference item:	Dinoterb (purity 98.0 % ± 0.5 analysed)
3. Test system:	
Soil:	Agriculturally utilised soil
Source:	Wassergut Canitz. field “Schlag 34/3”. Saxony. Germany
Water content of soil::	10.97 g/100 g soil d.w.
pH:	6.4
Total Org. C:	1.39 %
Clay (< 0.002 mm):	10.2 % (ISO 11277) / 10.2 % (USDA)
Silt (0.002-0.063 mm(ISO 11277) / (0.002-0.050(USDA)):	38.1 % (ISO 11277) / 36.8 % (USDA)
Sand (0.063 – 2.00 mm (ISO 11277) / 0.05-2.0 (USDA)):	51.7 % (ISO 11277) / 31.9 % (USDA)
4. Environmental conditions:	
Temperature:	19.5-21.1 °C
pH:	6.2-6.3
Water content:	41.15-43.00 % of WHC
Illumination	Darkness

B. STUDY DESIGN AND METHODS

1. Experimental treatments

For the investigation of potential effects of the test item Bulldock 25 EC. nitrogen transformation (NO3-nitrogen production) of test item treated soil was compared with a non-treated control soil. Per each replicate. 200 g soil d.w. per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal (the C/N ratio of the lucerne meal was 15.6/1). One additional soil sample (without lucerne meal) was used for determination of the initial NH4-N- and NO3- N-content. The NO3-N content was 1.82 mg/100 g soil d.w. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil. Two test rates of Bulldock 25 EC were applied: 0.96 mg test item/kg dry soil (corresponding to an application rate of 0.8 L test item/ha) and 9.61 mg test item/kg dry soil (corresponding to an application rate of 8 L test item/ha). Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50 % of WHC. Soil samples were incubated at 19.5-21.1 °C while stored in test vessels in the dark.

Although not required by OECD 216 the reference item Dinoterb was tested in a separate study (R 11 10 48 001 N) at concentrations of 6.8, 16.0 and 27.0 mg/kg.

2. Observations

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours. 7. 14 and 28 days after application and the NH_4-N. NO_3-N and NO_2-N content were determined. For extraction, 50 mL 1 M KCl solution (10 g soil d.w. with 50 mL KCl solution) and a rotator (150 rpm) were used. The extraction duration was 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at minus 20 ± 5 °C. The analysis was performed within one week after day 28. The pH-values of the soil were measured at test start (after application) and at the sampling on day 28, respectively. The limits of quantification for NO_3-N, NH_4-N and NO_2-N were 0.05 mg/100 g soil d.w. 0.06 mg/100

g soil d.w. and 0.1 mg/100 g soil d.w., respectively.

3. Statistical calculations
Mean values per treatment standard deviations and coefficients of variation were calculated.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

The validity criterion according to guideline OECD 216 requires a variation of less than ±15 % between replicate control samples for nitrogen transformation. The coefficients of variation in the control group of the nitrogen test were maximum 2.0 % and thus fulfilled the demanded range.
The results of the study are summarised in the tables below.

Table B.9.9-2: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	0.96 mg/kg dry weight soil equivalent to 0.8 L test item/ha		9.61 mg/kg dry weight soil equivalent to 8 L test item/ha	
	NO_3-N [mg/kg soil d.w.	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1]
0	20.47	20.07	-2.0	19.97	-2.4
7	30.03	30.77	+2.4	31.08	+3.3
14	38.73	37.63	-2.8	38.77	+0.1
28	48.83	48.23	-10.0	48.47	+10.6

The calculations were performed with non-rounded values
[1]based on NO_3-nitrogen production; - = inhibition; + = stimulation

Table B.9.9-3: Effects on nitrogen transformation in soil after treatment with Bulldock 25 EC. concentration change over time interval

Time Interval (days)	Control	0.96 mg/kg dry weight soil equivalent to 0.8 L test item/ha		9.61 mg/kg dry weight soil equivalent to 8 L test item/ha	
	NO_3-N/day [mg/kg soil d.w.	NO_3-N/day [mg/kg soil d.w.]	Deviation from control [%][1]	NO_3-N/day [mg/kg soil d.w.]	Deviation from control [%][1]
0-7	9.57	10.70	+11.8	11.07	+15.7
7-14	18.27	17.57	-3.8	18.80	+2.9
14-28	23.37	28.17	+20.5	28.50	+22.0

The calculations were performed with non-rounded values
[1]based on NO_3-nitrogen production; - = inhibition; + = stimulation

The reference item caused a stimulation of nitrogen transformation of 42.0 %. 68.1 % and 92.3 % at 6.8 mg. 16.0 mg and 27.0 mg Dinoterb per kg soil d.w., respectively, 28 days after application.

III. CONCLUSION

The study was performed in a field soil at concentrations equivalent up to an application rate of 8 L test item/ha. The test item caused no adverse effects (difference to control <25 %) on the soil nitrogen transformation (measured as NO_3-N production) 28 days after application.

B.9.10 Risk assessment for soil nitrogen transformation

Exposure
The maximum instantaneous predicted environmental concentrations of beta-cyfluthrin in soil (PECs) were calculated as described in Volume 3 CA Bulldock 25 B-8.

Table B.9.10-1: Predicted Environmental Concentrations in soil (PECs) of beta-cyfluthrin and soil metabolites FPB-acid and DCVA at 5 cm soil depth

Crop/Application rate [g as/ha]	Maximum instantaneous PECsoil [mg/kg]		
	Beta-Cyfluthrin	FPB-acid	DCVA
Wheat/2x7.5	0.014	0.003	0.004
Wheat/2x12.5	0.023	0.005	0.006
Potato/2x7.5	0.016	0.001	0.002
Potato/2x12.5	0.027	0.001	0.004

Bulldock 25 EC had no significant effect on soil micro-organisms at 9.61 mg Bulldock 25 EC /kg equivalent to 0.24 mg as/kg dry soil. This is approximately 8.9 times higher than the maximum PECs of 0.027 mg as/kg dry soil following the worst-case application to potatoes. This supports the conclusion that under field conditions the use of Bulldock 25 EC at the proposed rates poses no unacceptable risk to non-target soil micro-organisms. The metabolites FPB-acid and DCVA had no significant effect on soil micro-organisms at soil concentrations up to 0.125 mg/kg dry soil and 0.112 mg/kg dry soil, respectively. As this is 25 times higher for FPB-acid and approximately 18 times higher for DCVA than the maximum PECs no unacceptable effects are to be expected.

Table B.9.10-2: Risk on soil micro-organisms

Test substance	Endpoint	Value	Application rate (equivalent)	Max. field application rate of Bulldock 25 EC
Bulldock 25 EC	Nitrogen transformation	No negative effects up to 9.61 mg test item/kg dry soil (equivalent to 8 L test item/ha 200 g as/ha) after 28 days	200 g as/ha	2 x 0.5 L prod./ha (2x 12.5 g as/ha)

B.9.11 Effects on terrestrial non-target higher plants

Although beta-cyfluthrin is not a herbicide. limit tests on vegetative vigour and seedling emergence with the representative formulation Bulldock 25 EC were conducted to support the Approval of Renewal of beta-cyfluthrin.

A summary of the endpoints of the new studies conducted with Bulldock 25 EC is presented in Table B.9.11-1 below. Full details of these studies are provided in the following chapters.

Table B.9.11-1: Effects of Bulldock 25 EC to non-target terrestrial plants

Test substance Test type	Most sensitive species	Lowest ER_{50}	Reference
Bulldock 25 EC (formulated product) 21 d Seedling emergence	Green cabbage (*Brassica oleracea var. sabellica*) Cucumber (*Cucumis*	ER_{50} (phytotoxicity. seedling emergence. seedling fresh weight)	KIIIA1 10.8.1.3 Marquardt and Siemoneit-Gast. 2012a

	sativa) Carrot (*Daucus carota*) Lacy phacelia (*Phacelia tanacetifolia*) Sunflower (*Helianthus annuus*) Flax (*Linum usitatissimum*) Onion (*Allium cepa*) Rye grass (*Lolium multiflorum*) Barley (*Hordeum vulgare*) Erect brome (*Bromus erectus*)	> 60 g as/ha	M-438332-01-1 R-30155
Bulldock 25 EC (formulated product) 21 d Vegetative vigour	Green cabbage (Brassica oleracea var. sabellica) Cucumber (*Cucumis sativa*) Carrot (*Daucus carota*) Lacy phacelia (*Phacelia tanacetifolia*) Sunflower (*Helianthus annuus*) Flax (*Linum usitatissimum*) Onion (*Allium cepa*) Rye grass (*Lolium multiflorum*) Barley (*Hordeum vulgare*) Erect brome (*Bromus erectus*)	ER_{50} (phytotoxicity. seedling fresh weight) > 60 g as/ha	KIIIA1 10.8.1.2 Marquardt and Siemoneit-Gast. 2012b M-438396-01-1 R-30156

B.9.11.1 Summary of screening data

No studies on screening of non-target terrestrial plants are conducted and are not needed.

B.9.11.2 Testing on non-target plants

KIIIA1 10.8.1.3 (newly submitted with the dossier)

Author:	Marquardt. J.; Siemoneit-Gast. S.
Title:	Effect of Bulldock 25 EC on the seedling emergence of terrestrial plants.
Date:	15.08.2012
Doc ID:	M-438332-01-1
Report no.:	AS249
Edition no.:	R-30155
Guidelines:	OECD Guideline 208 (adopted July 2006)
GLP:	yes
Validity:	valid

Deviations:
The daily mean air humidity should be 70 % ± 25 %. The limit of 45 % was not maintained on three days after application (mean minimum 42 %). This deviation had no negative impact on the study. because the air humidity in the stand of plants was still within the range suited for a well growth of the plants.
The seedling emergence in the control should be at least 70 % at test termination. Due to a weak seedling emergence for Lacy phacelia the germination rate in the control was only 69 % at test

termination.
This deviation had no negative impact on the results of the study, because in the corresponding test rate 79 % of the sown seeds emerged after all. Therefore clear results could be obtained concerning a potential adverse effect of the test item to the test species Lacy phacelia uninfluenced from the weak seedling emergence in the control.
For the validation of the analytical method, five determinations are required (repeatability/precision). Instead, each preparation was only analysed in four replicates. This deviation had no negative impact on the result of the study, because four replicates are still sufficient to gain trustworthy results.

Dates of experimental work: 5 June 2012 to 17 July 2012

Executive Summary
A seedling emergence study was conducted exposing six dicotyledonous (green cabbage. cucumber. carrot. lacy phacelia. sunflower and flax) and four monocotyledonous species (onion. rye grass. barley and erect brome) according to OECD Guideline 208. The test rate was 2.4 L Bulldock 25 EC/ha (= 60 g active substance beta-cyfluthrin/ha). For each of the ten species. the test rate plus a water treated control were tested. Six replicate pots (containing five to ten seeds depending on the species) were prepared for both groups.
Assessments for seedling emergence and phytotoxicity were done 8. 14 and 21 days after application for all plants. The shoot fresh weight of the plant biomass above ground was determined at test termination (21 days after application) for all plants.
Bulldock 25 EC applied at 2.4 L/ha neither caused significant phytotoxicity nor effects on seedling emergence or plant fresh weight.
Accordingly. the NOER of Bulldock 25 EC was determined at the limit rate of 2.4 L Bulldock 25 EC/ha. corresponding to 60 g active substance/ha. for phytoxoxicity. seedling emergence and plant fresh weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC
Description:	Emulsifiable concentrate (EC)
Lot/Batch#:	92110164
Purity	active substance: beta-cyfluthrin (analysed active content: 24.9 g/L (w/w))

2. Reference item: None

3. Test organism:

Species:	6 dicotylenonous species: green cabbage (*Brassica oleracea* var. *sabellica*)[1]. cucumber (*Cucumis sativa*)[1]. carrot (*Daucus carota*)[1]. lacy phacelia (*Phacelia tanacetifolia*)[1]. sunflower (*Helianthus annuus*)[2] and flax (*Linum usitatissimum*)[3]. 4 monocotyledonous species: onion (*Allium cepa*)[1]. rye grass (*Lolium multiflorum*)[4]. barley (*Hordeum vulgare*)[5] and erect brome (*Bromus erectus*)[6].
Source:	[1] Hild Samen GmbH; [2] SUET Saat- und Erntetechnik GmbH; [3] Deutsche Saatveredelung;

[4] Meiners Saaten GmbH;
[5] KWS Lochow GmbH;
[6] Rieger-Hofmann GmbH.

4. Environmental conditions:

Temperature:	Daily mean temperature (min/max): 26 °C (25 °C / 28 °C)
Relative humidity:	Daily mean air humidity (min/max): 59 % (43 % / 79 %)
Photoperiod:	16 h light. 8 h dark; additional light when outdoor illumination was less than 5000 lux.
Soil pH [0.01 M $CaCl_2$]:	7.74
Soil organic carbon [% C]:	0.93

B. STUDY DESIGN

1. Experimental treatments

Six dicotyledonous (green cabbage. cucumber. carrot. lacy phacelia. sunflower and flax) and four monocotyledonous species (onion. rye grass. barley and erect brome) were exposed to the test item. Five to ten seeds (depending on the species) per each of the six replicates per species were sown two days before the application at different density and depth (species dependent) in the pots (13 cm inner diameter with bottom watering from a reservoir. filled with 875 g soil). The silty sand soil consisted of 6.2 % clay. 33.9 % silt and 59.9 % sand (pH 7.74; organic carbon 0.93 % C).
The pots were watered one day before the application. Bulldock 25 EC was applied pre-emergence in a volume equivalent to 300 L/ha using a laboratory spray cabin. It was applied at a rate of equivalent to 1.2 L/ha and application was repeated within 2 hours at the same rate (total 2.4 L/ha. nominal test rate. corresponding to 60 g active substance/ha). Deionised water served as control. The test plants were cultivated in the greenhouse at an average temperature of 26 °C. an average humidity of 59 % and a light:dark regime of 16:8 hours for 21 days (± 1 day).

2. Observations

The number of seedlings and the phytotoxicity were assessed 8,14 and 21 days after application for all plants. The phytotoxicity was rated in % affected plant volume per replicate compared to the control. One value for the sum of the considered parameters was obtained for each replicate, irrespective of the nature of symptoms. The assumed nature of symptoms was recorded. The type of the phytotoxic symptoms as well as their degree was judged only on the basis of visual observations. At test termination the plants were directly cut above the ground and the plant fresh weight per replicate was determined not later than 15 minutes after the cutting.

3. Statistical calculations

For phytotoxicity, seedling emergence and plant fresh weight, mean values and their standard deviations were calculated followed by analysis of variance (ANOVA) and by Student-t Test or Welch-t Test (α = 5 %). The NOER was defined as the tested rate if the tested rate did not cause a statistically significant effect compared to the control.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

There was no control mortality > 10 % observed and all control plants remained healthy throughout the complete test period. The rate of seedling emergence was ≥ 70 % for all tested plant species except lacy phacelia. For this species, the emergence rate was 69 % and thus only negligible lower compared to the control. Therefore, the study can be considered as valid.

Phytotoxicity: During the test, slight symptoms of phytotoxicity (on average < 10 %) were observed in the tested rate of 2.4 L test item/ha. The observed symptoms were growth reductions (green cabbage. cucumber. lacy phacelia. sunflower), deformations (green cabbage. sunflower) and chlorosis (carrot.

barley). However. the symptoms did not occur in every replicate or date of assessment and thus are not considered to be treatment-related. No symptoms were observed in flax, onion, rye grass and erect brome.

Seedling emergence: 8. 14 and 21 days after application the seedling emergence in the tested rate showed no statistically significant differences compared to the control or reductions in seedling emergence of ≥ 25 %.

Plant fresh weight: At test termination (21 days after application), the average plant fresh weight in the test rate was not statistically significantly different compared to the control.
Solely in lacy phacelia, the plant fresh weight in the test rate was significantly higher compared to the control. However, this was due to a better seedling emergence in the treatment group (79 %) compared to the control (69 %). The results are summarised below.

Table B.9.11-2: Effects on plants at test termination after pre-emergence application of Bulldock 25 EC

Plant species	Phytotoxicity		Seedling emergence		Plant fresh weight (shoots above ground)	
	NOER		NOER		NOER	
	L product/ha	g as/ha	L product/ha	g as/ha	L product/ha	g as/ha
Green cabbage (*Brassica oleracea var. sabellica*).	2.4	60	2.4	60	2.4	60
Cucumber (*Cucumis sativa)*	2.4	60	2.4	60	2.4	60
Carrot (*Daucus carota*)	2.4	60	2.4	60	2.4	60
Lacy phacelia (*Phacelia tanacetifolia*)	2.4	60	2.4	60	2.4	60
Sunflower (*Helianthus annuus*)	2.4	60	2.4	60	2.4	60
Flax (*Linum usitatissimum*)	2.4	60	2.4	60	2.4	60
Onion (*Allium cepa*)	2.4	60	2.4	60	2.4	60
Rye grass (*Lolium multiflorum*)	2.4	60	2.4	60	2.4	60
Barley (*Hordeum vulgare*)	2.4	60	2.4	60	2.4	60
Erect brome (*Bromus erectus*)	2.4	60	2.4	60	2.4	60

A concentration control analysis of the active substance beta-cyfluthrin in aqueous solution was performed by HPLC. The analysis of the test specimen yielded an analytical recovery of 100.5 %.

III. CONCLUSION

Bulldock 25 EC did not cause significant phytotoxicity or effects on seedling emergence or plant fresh weight in the ten tested plant species at the limit rate of 2.4 L/ha. Accordingly, the overall NOER is 2.4 L/ha Bulldock 25 EC, corresponding to 60 g active substance/ha, and the ER_{50} is assumed to be much higher than 2.4 L/ha Bulldock 25 EC.

KIIIA1 10.8.1.2 (newly submitted with the dossier)

Author:	Marquardt. J.; Siemoneit-Gast. S.
Title:	Effect of Bulldock 25 EC on the vegetative vigour of terrestrial plants.
Date:	15.08.2012

Doc ID:	M-438396-01-1
Report no.:	AS250
Edition no.:	R-30156
Guidelines:	OECD Guideline 208 (adopted July 2006)
GLP:	yes
Validity:	valid

Deviations:
The daily mean air humidity should be 70 % ± 25 %. The limit of 45 % was not maintained on three days after application (mean minimum 42 %). This deviation had no negative impact on the study. because the air humidity in the stand of plants was still within the range suited for a well growth of the plants.
For the validation of the analytical method, five determinations are required (repeatability/precision). Instead, each preparation was only analysed in four replicates. This deviation had no negative impact on the result of the study, because four replicates are still sufficient to gain trustworthy results.

Executive Summary

The objective of the study was to investigate the potential effect of the test item Bulldock 25 EC on the vegetative vigour of terrestrial plants. Six dicotyledonous and four monocotyledonous plant species were tested. An application rate of 1.2 L test item/ha was applied twice to mimic worst-case conditions of repeated application that can occur in the field. Thus, a total of equivalent 2.4 L test item/ha was applied to the plants in the pots. From each plant species two treatment groups (test item with the application rate + untreated control) were tested. Each treatment group consisted of six replicates. The test item was applied to the plants in a 2 to 4 leaf growth stage. After the repeated application, the plants were cultivated in the greenhouse for 21 days (± 1 day).
There was no control mortality > 10 % and all control plants remained healthy throughout the complete test period. Thus, the study can be considered as valid.
Bulldock 25 EC applied at 2.4 L/ha caused neither significant phytotoxicity nor effects on plant fresh weight.
Accordingly, the NOER of Bulldock 25 EC was determined at the limit rate of 2.4 L Bulldock 25 EC/ha corresponding to 60 g as/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Bulldock 25 EC
Description:	Emulsifiable concentrate (EC)
Lot/Batch#:	92110164
Purity	active substance: beta-cyfluthrin (analysed active content: 24.9 g/L (w/w))

2. Reference item: None

3. Test organism:

Species:	6 dicotylenonous species: green cabbage (*Brassica oleracea* var. *sabellica*)[1]. cucumber (*Cucumis sativa*)[1]. carrot (*Daucus carota*)[1]. lacy phacelia (*Phacelia tanacetifolia*)[1]. sunflower (*Helianthus annuus*)[2] and flax (*Linum usitatissimum*)[3].

	4 monocotyledonous species: onion (*Allium cepa*)[1]. rye grass (*Lolium multiflorum*)[4]. barley (*Hordeum vulgare*)[5] and erect brome (*Bromus erectus*)[6].
Source:	[1] Hild Samen GmbH; [2] SUET Saat- und Erntetechnik GmbH; [3] Deutsche Saatveredelung; [4] Meiners Saaten GmbH; [5] KWS Lochow GmbH; [6] Rieger-Hofmann GmbH.

4. Environmental conditions:

Temperature:	Daily mean temperature (min/max): 26 °C (25 °C / 28 °C)
Relative humidity:	Daily mean air humidity (min/max): 59 % (43 % / 79 %)
Photoperiod:	16 h light. 8 h dark; additional light when outdoor illumination was less than 5000 lux.
Soil pH [0.01 M $CaCl_2$]:	7.61
Soil organic carbon [% C]:	1.00

B. STUDY DESIGN AND METHODS

1. Experimental treatments

The potential effect of the test item Bulldock 25 EC on the vegetative vigour of six dicotyledonous and 4 monocotyledonous plant species was tested. The test item was applied at a rate of equivalent to 1.2 L/ha and application was repeated within two hours at the same rate (total 2.4 L/ha. nominal test rate). From each plant species two treatment groups (test item with the application rate + untreated control) were tested. Each treatment group consisted of six replicates. The test item was applied to the plants in a 2- 4 leaf growth stage. After the repeated application, the plants were cultivated in the greenhouse for 21 days (± 1 day) at 16:8 hours light:dark in soil consisting of loess. natural soil and quartz sand (pH: 7.61; organic carbon content: 1.0 % C). The average temperature in the greenhouse was 26 °C. the average humidity 59 % and the light:dark regime was 16:8 hours.

2. Observations

The phytotoxicity was assessed 7, 13 and 20 days after application for all plants. The phytotoxicity was rated in % affected plant volume per replicate compared to the control. One value for the sum of the considered parameters was obtained for each replicate irrespective of the nature of symptoms. The assumed nature of symptoms was recorded. The type of the phytotoxic symptoms as well as their degree was judged only on the basis of visual observations. At test termination the plants were directly cut above the ground and the plant fresh weight per replicate were determined not later than 15 minutes after the cutting.

3. Statistical calculations

For phytotoxicity and plant fresh weight, mean values and their standard deviations were calculated followed by analysis of variance (ANOVA) and by Student-t Test or Welch-t Test (α = 5 %). The NOER was defined as the tested rate. if the tested rate did not cause a statistically significant effect compared to the control.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

There was no control mortality > 10 % observed and all control plants remained healthy throughout the complete test period. Thus, the study can be considered as valid.

Phytotoxicity: No phytotoxicity was observed in green cabbage. cucumber. sunflower. flax. onion. rye grass. barley and erect brome. In carrots, slight symptoms of phytotoxicity were observed in two out of six replicates on day 13 and 20 (chlorosis. growth reduction). However, the average score was 2 %. In lacy phacelia, slight symptoms were seen in four out of six replicates, but only on day 20(average score 3 %). However, no phytotoxicity was seen at the end of the study. The effects in carrots and lacy phacelia are not considered to be clearly treatment-related.

Plant fresh weight: No statistically significant differences to the control or reductions in plant fresh weight of ≥ 25 % were observed in the tested species. In carrots. it was only slightly reduced in the test rate compared to the control. In onion and barley, plant fresh weight in the test rate was slightly increased. None of these slight effects were determined as statistically significant.

Table B.9.11-3: Effects on plants at test termination after post-emergence application of Bulldock 25 EC

Plant species	**Phytotoxicity**		**Plant fresh weight (shoots above ground)**	
	NOER		**NOER**	
	L product/ha	**g as/ha**	**L product/ha**	**g as/ha**
Green cabbage (*Brassica oleracea var. sabellica*).	2.4	60	2.4	60
Cucumber (*Cucumis sativa*)	2.4	60	2.4	60
Carrot (*Daucus carota*)	2.4	60	2.4	60
Lacy phacelia (*Phacelia tanacetifolia*)	2.4	60	2.4	60
Sunflower (Helianthus annuus)	2.4	60	2.4	60
Flax (*Linum usitatissimum*)	2.4	60	2.4	60
Onion (*Allium cepa*)	2.4	60	2.4	60
Rye grass (*Lolium multiflorum*)	2.4	60	2.4	60
Barley (*Hordeum vulgare*)	2.4	60	2.4	60
Erect brome (*Bromus erectus*)	2.4	60	2.4	60

A concentration control analysis of the active substance beta-cyfluthrin in aqueous solution was performed using HPLC method. The analysis of the test specimen yielded an analytical recovery of 104.6 %.

III. CONCLUSION

Bulldock 25 EC did neither cause phytotoxicity nor effects on plant fresh weight at the limit rate of equivalent 2.4 L Bulldock 25 EC /ha. Thus, the overall NOER is 2.4 L/ha Bulldock 25 EC corresponding to 60 g as/ha, and the ER_{50} is assumed to be much higher than 2.4 L/ha Bulldock 25 EC.

B.9.11.3 Extended laboratory studies on non-target plants

Bulldock 25 EC is an insecticide and is therefore not expected to have herbicidal activity. The limit tests at an exaggerated rate of 2.4 L/ha Bulldock 25 EC did not show any significant effects. confirming the absence of herbicidal properties. Accordingly, the studies on seedling emergence and vegetative vigour are sufficient for risk assessment, and extended laboratory studies on non-target plants are not required.

B.9.11.4 Semi-field and field tests on non-target plants

Bulldock 25 EC is an insecticide and is therefore not expected to have any significant herbicidal activity. This was confirmed in studies on seedling emergence and vegetative vigour. Accordingly, no semi-field and field tests on non-target plants are required.

B.9.12 Risk assessment for terrestrial non-target higher plants

Bulldock 25 EC was tested at a rate of 2.4 L/ha (60 g as/ha. limit test) with 10 different species representing different plant taxa. Endpoints investigated were phytotoxicity. plant fresh weight and seedling emergence in the respective test. The treatment did not cause any effects in any of the plants. Accordingly, the NOER was 2.4 L/ha Bulldock 25 EC (60 g as/ha) and the ER_{50} > 2.4 L/ha Bulldock 25 EC (60 g as/ha).

Exposure

Effects on non-target plants are of concern in the off-field environment. where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the *BBA (2000)* [7] from the spray-drift predictions of *Ganzelmeier & Rautmann (2000)*[8]. For a single application to field crops. 2.77 % of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of Bulldock 25 EC is 0.5 L product/ha in wheat and potatoes. giving a maximum off-field predicted environmental rate ($PER_{off\text{-}field}$) of 13.85 mL product/ha. equivalent to 0.346 g as/ha.

Risk assessment

Bulldock 25 EC is an insecticide and is therefore not expected to have any significant herbicidal activity. Studies on possible pre- and post-emergence effects on non-target higher plants showed no effects on any of the species tested at a limit rate of 2.4 L Bulldock 25 EC/ha. The calculated maximum $PER_{off\text{-}field}$ of 13.85 mL product/ha (wheat and potatoes) equivalent to 0.346 g as/ha is far below the level found to have no effects on non-target plants. The resulting TER values are given in Table B.9.12-1 below.

Table B.9.12-1: Bulldock 25 EC: TERs for 10 terrestrial non-target terrestrial plants based on PERoff-field and ER_{50} from a 21 d vegetative vigour test and 21 d seedling emergence test (>60 g as/ha)

Crop	Application rate [g as/ha]	Maximum drift at 1 m distance (%)	Off-field drift rate ($PER_{off\text{-}field}$) [g as/ha]	Endpoint [g as/ha]	TER	Trigger value
Wheat.potato	7.5	2.77	0.208	>60	>289	5
Wheat. potato	12.5	2.77	0.346	>60	>173	5

Bulldock 25 EC will not pose a risk to non-target terrestrial plants because realistic exposure rates are far below the No Observed Effect Rate in both vegetative vigour and seedling emergence test. TERs based on maximum drift at 1 m distance significantly exceed the trigger of 5.

B.9.13 Effects on other terrestrial organisms (flora and fauna)

No further studies on effects on other terrestrial organisms are required.

B.9.13.1 Risk assessment for other terrestrial organisms (flora and fauna)

Not applicable.

B.9.14 References relied on

In every chapter (B.1. B.2. etc.) in Volume 3 (AS) the reference relied on heading should start with a paragraph indicating how the literature search was carried out and if this is considered acceptable. It should also be indicated if the RMS can agree with the justifications given by the notifier (especially for non-relevant literature).This is not expected to be a detailed study-by-study consideration. Relevant literature would be evaluated and assessed in the normal way within each section.

For (draft) renewal assessment reports the reference lists at the end of each section/chapter (sorted by data requirement) should include the newly submitted data relied upon as well as those original submitted tests and studies that are still considered relevant to support the application for renewal. However these studies should be clearly identified in the reference list as well as in the individual study sections. This could be done by consistent use of a statement for each study:
Previous evaluation: responded “N.A.” for NAS. “Submitted for the purpose of renewal”. or “In DAR (year)”. “In addendum to DAR (year)” or any other appropriate

B.10 Reference list of non-vertebrate studies sorted by Annex point

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.2	Grace. Nillos Mae. Qin Sujie. Larive Cynthia. Schlenk Daniel. Gan Jay.	2009	Epimerisation of cypermethrin stereoi-somers in alcohols. Journal of agricultural and food chemis-try 57 (15): 6938-43. doi:10.1021/jf900921g. published	N	N		LIT
KIIIA1 10.2	Perschke. H.; Hussain. M.	1992	Chemical isomerisation of deltamethrin in alcohols. J. Agric. Food Chem. 1992. 40. 686–690. published	N	N		LIT
KIIIA 10.2.2.2/01	**Bruns. E.**	**2010**	**Acute toxicity of beta-Cyfluthrin EC25A G to the waterflea Daphnia magna in a static renewal laboratory test system** **Report No.: EBFRL008** **Edition No.: M-372834-01-1; R-28699** **Bayer CropScience AG. Monheim. Germany** **GLP** **not published**	**N**	**Y**	**data not submitted on EU level**	**BCS/ IRV**

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.2.6/01	Heimbach. F.	2000	Comparative toxicity of ^{14}C-Cyfluthrin EC 050 to Gammarus pulex in water and in a water sediment system under static laboratory conditions Report No: HBF/SP 01-99 Edition No.: M-020399-01-1; R-19104 GLP not published	N	N	-	BCS
KIIIA1 10.2.2.3	Heimbach. F.	1988	Growth inhibition of green algae (Scenedesmus subspicatus) by FCR 4545 EC 025 Report No: M-055550-01-1 Edition No.: R-19160 GLP not published	N	N	-	BCS
KIIIA1 10.2.6/01	Heimbach. F.	1999	Extended laboratory study on effects and recovery of a Daphnia magna population in a water-sediment system after application of 14C-Cyfluthrin EC 050 [redacted] Report No: HBF/EDM 04 Edition: M-041214-01-1; R-19100 BAY GLP not published	N	N	-	BCS
KIIIA1 10.2.3/01	Heimbach. F.	1989	Biological effects and fate of FCR 4545 EC 025 (R Bulldock) in experimental ponds Report no.: HBF/VT 01 Edition no.: M-059813-01-1; R-19093 (in baseline saved as M-022657-01-1) Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.2.3/02	Heimbach. F.	1990	Biological effects and fate of FCR 4545 EC 025 (Bulldock) in artificial ponds Report No: HBF/MT 01 Edition no.: M-059808-01-1; R-19094 (in baseline saved as M-022640-01-1) GLP not published	N	N	-	BCS
KIIIA1 10.2.3/03	Heimbach. F.	2000	Biological effects and fate of Cyfluthrin EC 050 in outdoor microcosm ponds Report No: HBF/BT 02 Edition No.: M-029184-01-1. R-19090a BCS GLP not published	N	N	-	BCS
KIIIA1 10.2.3/05	Jenkins. W.R.	2014	Beta-cyfluthrin (Bulldock 25 EC): toxicity to *Asellus. Crangonyx. Chaoborus* and *Cloeon* in outdoor microcosms Report no.: JDV0118 Edition no.: R-34676 GLP not published	N	Y		BCS. IRV
KIIIA1 10.2.3/04	Hommen. U.. Heimbach. F.	2000	Evaluation of an outdoor microcosm study on Cyfluthrin (report no. HBF/Bt 02 of March 15. 2000) for an aquatic risk assessment Report No: HBF/BT 02a Edition No.: M-032767-01-1; R-19090b Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.1.1/04	Schmitzer. S.. Sekine. T.	2010	Effects of beta-Cyfluthrin EC 025 G (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory Report No.: 52601035 Edition No.: M-363013-01-1; R-30619 IBACON. Rossdorf. Germany GLP not published	N	Y	to complete the risk assessment for bees using the current representative formulation	IRV/ BCS
KCP 10.3.1.1/01	Pinsdorf. W.	1987	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit - Versuchsplan 1987- Firmenauftrag (Laboratoriumspruefung) Report No: B-87281 Edition No.: M-054264-01-1; R-19117 Not GLP not published	N	N	-	BCS
KCP 10.3.1.1/02	Stute. K.	1987	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit 1987 - Laboratoriumspruefung Report No: B-87280 Edition No.: M-054276-01-1; R-19118 Not GLP not published	N	N	-	BCS
KCP 10.3.1.1/03	Mautz	1987	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Laboratoriumspruefung Report No: 870185 Edition No.: M-054254-01-1; R-19116 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.1/04	Kleiner. R.	1996	Testing toxicity to honeybee - Apis mellifera L. (laboratory) according to EPPO Guideline No. 170 (1992) - FCR 4545 Report No: M-053813-01-1 GLP not published	N	N	-	BCS
KCP 10.3.1.2	Sandrock, C.	2014	Bulldock 25 EC: Toxicity effects to honey bee (Apis mellifera L.) worker adults after oral chronic exposure under laboratory conditions Report No.: 20120186; M-479053-01-1 GLP not published	N	J		IRV/ BCS
KCP 10.3.1.2	Sandrock, C.	2014	Bulldock 25 EC: Toxicity effects to honey bee (Apis mellifera L.) larvae after single exposure under laboratory conditions Report No.: 20120187; M-479050-01-1 GLP not published	N	J		IRV/ BCS
KCP 10.3.1.5/01	Schulz. A.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890901 Edition No.: M-054119-01-1 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.5/02	Schulz. A.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890902 Edition No.: M-053980-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/03	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890300 Edition No.: M-054240-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/04	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890301 Edition No.: M-054235-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/05	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890302 Edition No.: M-054232-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/06	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890303 Edition No.: M-054228-01-1 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.5/07	Pinsdorf. W.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890622 Edition No.: M-054212-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/08	Pinsdorf. W.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890623 Edition No.: M-054209-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/09	Pinsdorf. W.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890624 Edition No.: M-054153-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/10	Pinsdorf. W.	1989	Pruefung auf Bienengefarhrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 890625 Edition No.: M-054146-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.5/11	Stute. K.	1987	Pruefung auf Bienengefarhrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 870248 Edition No.: M-054250-01-1 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.5/12	Stute. K.	1987	Pruefung auf Bienengefarhrlichkeit fuer das Zulassungsverfahren - Zeltpruefung Report No: 870249 Edition No.: M-054246-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/01	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Freilandpruefung Report No: 890304 Edition No.: M-054102-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/02	Stute. K.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Freilandpruefung Report No: 890305 Edition No.: M-054094-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/03	Pinsdorf. W.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Freilandpruefung 1989 Report No: 890620 Edition No.: M-053915-01-1 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.6/04	Pinsdorf. W.	1989	Pruefung auf Bienengefaehrlichkeit fuer das Zulassungsverfahren - Freilandpruefung 1989 Report No: 890621 Edition No.: M-053897-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/05	Stute. K.	1985	Result of the official examination of bee toxicity for registration - 1985 - Field test Report No: B-85545 Edition No.: M-008797-01-2 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/06	Stute. K.	1985	Result of the official examination of bee toxicity for registration - 1985. field test Report No: B-85550 Edition No.:M-008811-01-2 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/07	Stute. K.	1986	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit 1986a - Freilandpruefung Report No: B-86539 Edition No.: M-031446-01-1 Not GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.6/08	Stute. K.	1986	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit 1986 b - Freilandpruefung Report No: B-86540 Edition No.: M-031449-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/09	Stute. K.	1986	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit 1986 - Freilandpruefung Report No: B-86543 Edition No.: M-031485-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/10	Stute. K.	1986	Ergebnis der Zulassungspruefung auf Bienengefaehrlichkeit 1986 - Freilandpruefung Report No: B-86544 Edition No.: M-031492-01-1 Not GLP not published	N	N	-	BCS
KCP 10.3.1.6/11	Nengel. S.	1997	Assessment of effects of Bulldock EC 025 on the honey bee (Apis mellifera L.) in the field following application during bee-flight Report No: 97152/01-BFEU Edition No.: M-022622-01-1 GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.6/12	Kleiner. R.	1998	Testing toxicity to honeybee - Apis mellifera L. (under field conditions) according to BBA Guideline VI. 23-1 (1991) Report No: 971048049 Edition Number: M-022631-01-1 GLP not published	N	N	-	BCS
KCP 10.3.1.6/13	Sandrock. C	2014c	Bulldock 25 EC (as: beta-Cyfluthrin) - A Field Study to Evaluate Potential Side Effects on Brood Development. Foraging Activity. Mortality and Behaviour of Adult Honeybees. Apis mellifera L. (Hymenoptera: Apidae). Following the Application after Bee-Flight on Phacelia tanacetifolia in 2013. Report no: 20130101 Edition number: R-33347 Innovative Environmental Services (IES) Ltd. Switzerland GLP not published	N	Y	high tier study to complete the risk assessment for bees	IRV

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KCP 10.3.1.6/14	Sandrock. C	2014d	Bulldock 25 EC (as: beta-Cyfluthrin) - A Field Study to Evaluate Potential Side Effects on Brood Development. Foraging Activity. Mortality and Behaviour of Adult Honeybees. Apis mellifera L. (Hymenoptera: Apidae). Following the Application after Bee-Flight on Phacelia tanacetifolia. Report no: 20120046 Edition number: R-28685 Innovative Environmental Services (IES) Ltd. Switzerland GLP not published	N	Y	high tier study to complete the risk assessment for bees	IRV
KIIIA1 10.5.1/01	**Roig. J.**	**2014**	**A Tier 1 laboratory dose-response study to assess the LR50 of Bulldock 25 EC for the parasitoid wasp Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera: Braconidae) in ventilated glass cages** **MITOX Consultants. Amsterdam. Netherlands** **BCS-Irvita.** **Report No.: FC011ARL.** **Edition Number:** M-479582-01-1 **Date: 2014-03-03** **GLP/GEP: yes. unpublished**	**N**	**Y**	**to complete the data package**	**BCS-Irvita**

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.1/02	**Roig. J.**	**2014**	**Report Amendment no. 1 - A tier 1 laboratory dose-response study to assess the LR50 of Bulldock 25 EC for the evaluate the predaceous mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) in ventilated glass cages MITOX Consultants. Amsterdam. Netherlands BCS-Irvita. Report No.: FC010TPL. Edition Number:** M-479587-02-1 **Date: 2014-03-03 ...Amended: 2014-03-25 GLP/GEP: yes. unpublished**	**N**	**Y**	**to complete the data package**	**BCS-Irvita**
KIIIA1 10.5.1/03	Heimbach. F.	1990	Toxicity of Bulldock (025 EC) to carabid beetles (Poecilus cupreus) Report No: M-052707-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.1./04	Schmuck. R.	1992	Effects of Bulldock EC 025 on the life cycle of rove beetles (Aleochara bilineata) under laboratory conditions Report No: M-052616-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.1./05	Kuehner. C.	1993	Assessment of side effects of BAY 13210 I on the green lacewing. Chrysoperla carnea Steph. in the laboratory Report No: M-052746-01-2 GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.2./01	Kleiner. R.	1997	Testing toxicity to beneficial arthropods cereal aphid parasitoid - Aphidius rhopalosiphi (Destefani - Perez) / pupae according to IOBC guideline (Mead-Briggs 1992) Report No: M-052305-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.2./02	Neumann. P.	2001	Acute effects of beta-Cyfluthrin EC 025 on larvae of carabid beetles (Poecilus cupreus) under extended laboratory test conditions Report No: M-080415-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.2/03	Aldershof. S. A.	1999	Bulldock EC025: An extended laboratory dose-response study to evaluate the effects on the predaceous mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) on detached apple leaves Report No: M-022573-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.2./04	Kleiner. R.	2001	Beta-cyfluthrin EC 025 - Toxicity to larvae of Coccinella septempunctata l. under extended laboratory conditions Report No: M-032166-01-1 GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.2./05	Neumann. P.	2000	Effects of beta-Cyfluthrin EC 025 on the ladybird beetle (Coccinella septempunctata) under extended laboratory conditions (aged residue test) Report No: NNP/CS004 Edition No.: M-038111-01-1; R-19205 GLP not published	N	N	-	BCS
KIIIA1 10.5.2./06	Aldershof. S. A.	1999	Bulldock EC025: An extended laboratory dose-response study to evaluate the effects on the parasitoid Aphidius rhopalosiphi on detached apple leaves Report No: M-015321-01-1 GLP not published	N	N	-	BCS
KIIIA1 10.5.2./07	**Moll. M.**	**2004a**	**Effects of Bulldock EC 025 on the Ladybird Beetle Coccinella septempunctata. Extended Laboratory Study – Aged Residue Test – Spray Drift Application Rates** **Report No.: 18121013** **Edition No.: R-19424** **IBACON GmbH. Rossdorf. Germany** **GLP** **not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	IRV

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.2./08	**Moll. M.**	**2004b**	**Effects of Bulldock EC 025 on the Ladybird Beetle Coccinella septempunctata. Extended Laboratory Study – Aged Residue Test – Field Application Rate** **Report No.: 18122013** **Edition No.: R-19425** **IBACON GmbH. Rossdorf. Germany** **GLP** **not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	IRV
KIIIA1 10.5.2./09	**Moll. M.**	**2005a**	**Effects of Bulldock EC 025 on the Ladybird Beetle Coccinella septempunctata. Extended Laboratory Study – Aged Residue Test** **Report No.: 25141013** **Edition No.: R-19594** **IBACON GmbH. Rossdorf. Germany** **GLP** **not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	IRV
KIIIA1 10.5.2./10	**Moll. M.**	**2005b**	**Effects of Baythroid 050 EC on the Ladybird Beetle Coccinella septempunctata. Extended Laboratory Study – Aged Residue Test** **Report No.: 25151013** **Edition No.: R-19593** **IBACON GmbH. Rossdorf. Germany** **GLP** **not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	**IRV**

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.3/01	**Schmuck. R.**	**1993a**	**Acute effects of a spray application of Bulldock EC 025 on carabid beetles (Poecilus cupreus) under semifield conditions Report No: SXR/HF 63 Edition No.: M-052352-01-1; R-19131 BCS Not GLP not published**	**N**	**N**	**-**	**BCS**
KIIIA1 10.5.3/02	Schmuck. R.	1993b	Acute effects of a spray application of Bulldock EC 025 on carabid beetles (Poecilus cupreus) under semifield conditions Report no.: SXR/HF 88 Edition no.: M-052490-01-1; R-19130 BCS GLP not published	N	N	-	BCS
KIIIA1 10.5.3/03	Schmuck. R.	1993c	Acute effects of a spray application of Bulldock EC 025 on carabid beetles (Poecilus cupreus) under semifield conditions Report no.: SXR/HF 91 Edition no.: M-052542-01-1; R-19129 BCS GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.3/04	Schmuck. R.	1992	Acute Effects of a spray application of Bulldock EC 025 on carabid beetles under semifield conditions Report no.: SXR/HF 56 Edition no.: M-052680-01-1; R-19138 BCS GLP not published	N	N	-	BCS
KIIIA1 10.5./01	Barrett. K. L.. Grandy. N.. Harrison. E. G.. Hassan. S.. Oomen. P.	1994	Guidance document on regulatory testing procedures for pesticides with non-target arthropods Report No: M-001914-01-1 SETAC Not GLP published	N	N	-	-public data-
KIIIA1 10.5.3/01	Redl. H.. Fuchs. A.	1992	Auswirkungen einer Austriebsbehandlung von Reben auf den Raubmilbenbesatz Report No: Lit. 6738 Edition No.: M-090498-01-1; R-19137 Not GLP Published: Mitteilungen Klosterneuburg. Volume:42. Pages:228-230	N	N	-	-public data-

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.3/02	Darwish. Y. A.. Farghal. A. I.	1990	Evaluation of certain pesticides activity against the cotton whitefly. bemesia tabaci and associated natural enemies on cotton plants under field conditions in Assiut (Egypt) Report No: Lit. 7098 Edition No.: M-090543-01-1; R-19134 Not GLP Published: Journal of Agricultural Sciences. Volume:21. Issue:5. Pages:331-339	N	N	-	-public data-
KIII1 10.5./02	Candolfi. M. P.. Barrett. K. L.. Campbell. P. J.. Forster. R.. Grandy. N.. Huet. M. C.. Lewis. G.. Oomen. P. A.. Schmuck. R.. Vogt. H.	2001	Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods Report No: M-083833-01-1 BAY Not GLP published	N	N	-	-public data-
KIIIA1 10.5.3/03	**Vinall. S.**	**2005**	**A field trial to determine the effects of Baythroid EC 050 (50 g/L Cyfluthrin) on the non-target arthropod fauna of a winter-sown cereal crop. following two applications during spring/summer** **Report No.: IRV-04-1** **Edition No.: R-19598** **Mambo-Tox Ltd. 2 Venture Road. Chilworth Science Park. Southampton SO16 7NP** **GLP** **not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	**IRV**

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.4/04	Vinall. S.	2006	A field trial to determine the effects of Baythroid EC 050 (50 g/L Cyfluthrin) on the non-target arthropod fauna of an orchard crop. following two applications during spring/summer Report No.: IRV-05-1 Edition No.: R-19592 Mambo-Tox Ltd. 2 Venture Road. Chilworth Science Park. Southampton SO16 7NP GLP not published	N	Y	higher tier study to complete the risk assessment for non-target arthropods	IRV
KIIIA1 10.5.3/05	Mack P.	2013	A field study assessing the impact of Bulldock 25 EC on the non-target arthropod fauna in an alfalfa field in Spain Report No.: S12-01037 Edition No.: R-28693 Eurofins Agroscience Services. EcoChem GmbH. Eutinger Str. 24. 75223 Niefern-Öschelbronn. Germany GLP not published	N	Y	higher tier study to complete the risk assessment for non-target arthropods	IRV

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.5.3/06	**Knäbe. S.**	**2013**	**A field study assessing the impact of Bulldock 25 EC on the non-target arthropod fauna in pome fruit orchard in Germany Report No.: S12-01040 Edition No.: R-28694 Eurofins Agroscience Services. EcoChem GmbH. Eutinger Str. 24. 75223 Niefern-Öschelbronn. Germany GLP not published**	**N**	**Y**	**higher tier study to complete the risk assessment for non-target arthropods**	**IRV**
KIIIA1 10.5.3/07	**Mack P.**	**2014**	**A Field Study Assessing the Impact of Drift Rates of beta-cyfluthrin on the Non-Target Arthropod Fauna on a Meadow in Germany Report No.: S13-00176 Edition No.: R-30607 Eurofins Agroscience Services. EcoChem GmbH. Eutinger Str. 24. 75223 Niefern-Öschelbronn. Germany GLP not published**	**N**	**Y**	**to complete the risk assessment for non-target arthropods**	**IRV**
KIIIA1 10.6.2	Heimbach. F.	1988	Acute toxicity of FCR 4545 EC 025 to earthworms Report No: M-053588-01-2 GLP not published	N	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.6.3	**Pavic. B.**	**2013**	**Effects of Bulldock 25 EC on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10 percent peat** **IBACON GmbH. Rossdorf. Germany** **Irvita Plant Protection.** **Report No.: 74484022.** **Edition Number:** M-461275-01-1 **Date: 2013-07-03** **GLP/GEP: yes. unpublished**	**N**	**Y**	**New data requirement**	**BCS-Irvita**
KIII1 10.6.6	**McCormac. A.**	**2014**	**Bulldock 25 EC – A laboratory test to determine the effects of fresh residues on the springtail Folsomia candida (Collembola. Isotomidae)** **Report No.: IRV-13-7** **Edition No.: R-33352** **Mambo-Tox Ltd.. Southampton. UK** **GLP** **not published**	**N**	**Y**	**to complete the risk assessment for non-target soil macroorganisms**	**IRV**
KIIIA1 10.7.1	**Schulz. L.**	**2011**	**Bulldock 25 EC – Effects on the activity of soil microflora (Nitrogen test)** **Report No.: 11 10 48 084 N** **Edition No.: R-28684** **BioChem Agrar. Gerichshain. Germany** **GLP** **not published**	**N**	**Y**	**to complete the risk assessment for non-target soil microorganisms**	**IRV**

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.8.1.3	**Marquardt. J.. Siemoneit-Gast. S.**	**2012**	**Effect of Bulldock 25 EC on the seedling emergence of terrestrial plants. Report No.: AS249 Edition No.: M-438332-01-1; R-30155 Rheinland-Pfalz (RLP) AgroScience GmbH. Neustadt an der Weinstraße. Germany. GLP not published**	**N**	**Y**	**to complete the risk assessment for non-target terrestrial plants**	**BCS/ IRV**
KIIIA1 10.8.1.2	**Marquardt. J.. Siemoneit-Gast. S.**	**2012**	**Effect of Bulldock 25 EC on the vegetative vigour of terrestrial plants. Report No.: AS250 Edition No.: M-438396-01-1; R-30156 Rheinland-Pfalz (RLP) AgroScience GmbH. Neustadt an der Weinstraße. Germany. GLP not published**	**N**	**Y**	**to complete the risk assessment for non-target terrestrial plants**	**BCS/ IRV**

IRV = Irvita Plant Protection. Curacao – a member of Makhteshim Agan Holding B.V.. The Netherlands
BCS = Bayer CropScience AG. Monheim. Germany
Grey = Studies were discussed in Volume 3. but were not used in the risk assessment.
Black = Studies used in the risk assessment
Bold = Studies submitted for the first time in support of the renewal approval of beta-Cyfluthrin
Yellow underlined = studies used by JKI (can be deleted)
Red = guidance documents

B.11 Reference list of vertebrate studies sorted by Annex point

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA 10.1.6	[redacted]	**2010**	**Acute Oral Toxicity to Northern Bobwhite Quail (Colinus virginianus) of Beta Cyfluthrin EC 025G** **Report no.: BAR/LD113** **Edition number: M-367618-01-1; R-30618** [redacted] **GLP** **not published**	**Y**	**Y**	**to complete the risk assessment for birds using the current representative formulation**	**BCS/ IRV**
KIIIA1 10.2.2.1	[redacted]	1989	Acute toxicity of bulldock to rainbow trout (Salmo gairdneri) in a flow-through test [redacted] Report No: M-055191-01-2. R-19157 GLP not published	Y	N	-	BCS
KIIIA1 10.2.2.1/02	[redacted]	1989	Acute toxicity of Bulldock to golden orfe (Leuciscus idus melanotus) in a flow-through test Report No: M-055138-01-2 GLP not published	Y	N	-	BCS

Annex point/ reference number	Author(s)	Year	Title Report No. Source (where different from company) GLP status (where relevant) published or not	Vertebrate study	Data protection claimed	Justification	Owner
KIIIA1 10.2.3/06	[redacted]	1986	Baythroid - Pond study Report No.: F-867962 Edition No.: M-059891-01-1; R-19091 [redacted] Not GLP not published	Y	N	-	BCS
KIIIA1 10.2.2.1/3	[redacted]	**2005**	**Beta-cyfluthrin (Bulldock 025 EC). Toxicity to Rainbow trout Oncorhynchus mykiss in outdoor microcosms** [redacted] **Report No.: IRV 118/053116** **Edition No: R-19589** **GLP** **not published**	**Y**	**Y**	**higher tier study to support chronic fish risk assessment**	**IRV**

IRV = Irvita Plant Protection. Curacao – a member of Makhteshim Agan Holding B.V.. The Netherlands
BCS = Bayer CropScience AG. Monheim. Germany
Grey = Studies were discussed in Volume 3. but were not used in the risk assessment.
Black = Studies used in the risk assessment
Bold = Studies submitted for the first time in support of the renewal approval of beta-Cyfluthrin

B.11.1 References of Guidance documents and open literature:

Annex point / reference number	citation
KIIIA1 10.1	European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu
KIIIA1 10.2	Grace. Nillos Mae. Qin Sujie. Larive Cynthia. Schlenk Daniel. Gan Jay. 2009. "Epimerisation of cypermethrin stereoisomers in alcohols." Journal of agricultural and food chemistry 57 (15): 6938-43. doi:10.1021/jf900921g.
KIIIA1 10.2	Perschke. H.; Hussain. M. Chemical isomerisation of deltamethrin in alcohols. J. Agric. Food Chem. 1992. 40. 686–690.
KIIIA1 10.2	European Food Safety Authority; Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters on request from EFSA. EFSA Journal 2013;11(7):3290
KIIIA1 10.5	de Jong. F.M.W. Bakker. F.M.. Brown. K.. Jilesen. C.J.T.J.. Posthuma-Doodeman. C.J.A.M.. Smit. C.E.. van der Steen. J.J.M. van Eekelen. G.m.A. 2010. Guidance for summarising and evaluating field studies with non-target arthropods - A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. Published by the National Institute for Public Health and the Environment. Available online: ww.rivm.nl/bibliotheek/601712006/pdf
KIIIA1 10.5	Candolfi. M.P.. Barrett. K.L.; Campbell. P.J.. Forster. R.. Grandy. N.. Huet. M-C. Lewis. G. Oomen. P.A.. Schmuck. R. and Vogt. H. 2000.".Guidance document on regulatory testing and risk assessment procedures for plant protectionproducts with non-target arthropods – From the ESCORT 2 workshop"
KIIIA1 10.6	J Boesten. A Helweg. M Businelli. L. Bergstrom. H Schaefer. A Delmas. R Kloskowski. A Walker. K Travis. L Smeets. R Jones.V Vanderbroeck. A Van Der Linden. S Broerse. M Klein. R Layton. O-S Jacobsen. D Yon. 1997. "Soil persistence models and EU registration - The final report of the work of the Soil Modelling Work group of FOCUS" Available online: http://ec.europa.eu/food/plant/protection/evaluation/guidance/soil_en.pdf
	Guidance document (GD) for terrestrial ecotoxicology (SANCO/10329/2002